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(54) Title: MAMMALIAN EXPRESSION SYSTEMS FOR HCV PROTEINS (57) Abstract Mammalian expression systems for the production of HCV proteins. Such expression systems provide high yields of HCV proteins, and enable the development of diagnostic and therapeutic reagents which contain glycosylated structural antigens and also allow for the isolation of the HCV etiological agent.		

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MAMMALIAN EXPRESSION SYSTEMS FOR HCV PROTEINS

Background of the Invention

This invention relates generally to Hepatitis C Virus (HCV), and more particularly, relates to mammalian expression systems capable of generating HCV proteins and uses of these proteins.

Descriptions of Hepatitis diseases causing jaundice and icterus have been known to man since antiquity. Viral hepatitis is now known to include a group of viral agents with distinctive viral organization protein structure and mode of replication, causing hepatitis with different degrees of severity of hepatic damage through different routes of transmission. Acute viral hepatitis is clinically diagnosed by well-defined patient symptoms including jaundice, hepatic tenderness and an elevated level of liver transaminases such as Aspartate Transaminase and Alanine Transaminase.

Serological assays currently are employed to further distinguish between Hepatitis-A and Hepatitis-B. Non-A Non-B Hepatitis (NANBH) is a term first used in 1975 that described cases of post-transfusion hepatitis not caused by either Hepatitis A Virus or Hepatitis B Virus. Feinstone et al., New Engl. J. Med. 292:454-457 (1975). The diagnosis of NANBH has been made primarily by means of exclusion on the basis of serological analysis for the presence of Hepatitis A and Hepatitis B. NANBH is responsible for about 90% of the cases of post-transfusion hepatitis. Hollinger et al. in N. R. Rose et al., eds., Manual of Clinical Immunology, American Society for Microbiology, Washington, D. C., 558-572 (1986).

Attempts to identify the NANBH virus by virtue of genomic similarity to one of the known hepatitis viruses have failed thus far, suggesting that NANBH has a distinctive genomic organization and structure. Fowler et al., J. Med. Virol. 12:205-213 (1983), and Weiner et al., J. Med. Virol. 21:239-247 (1987). Progress in developing assays to detect antibodies specific for NANBH has been hampered by difficulties encountered in identifying antigens associated with the virus. Wards et al., U. S. Patent No. 4,870,076; Wards et al., Proc. Natl. Acad. Sci. 83:6608-6612 (1986); Ohori et al., J. Med. Virol. 12:161-178 (1983); Bradly et al., Proc. Natl. Acad. Sci. 84:6277-6281 (1987); Akatsuka et al., J. Med. Virol. 20:43-56 (1986).

In May of 1988, a collaborative effort of Chiron Corporation with the Centers for Disease Control resulted in the identification of a putative NANB agent, Hepatitis C Virus (HCV). M. Houghton et al. cloned and expressed in E. coli a NANB

agent obtained from the infectious plasma of a chimp. Cuo et al., Science 244:359-361 (1989); Choo et al., Science 244:362-364 (1989). CDNA sequences from HCV were identified which encode antigens that react immunologically with antibodies present in a majority of the patients clinically diagnosed with NANBH.

- 5 Based on the information available and on the molecular structure of HCV, the genetic makeup of the virus consists of single stranded linear RNA (positive strand) of molecular weight approximately 9.5 kb, and possessing one continuous translational open reading frame. J. A. Cuthbert, Amer. J. Med. Sci. 299:346-355 (1990). It is a small enveloped virus resembling the Flaviviruses. Investigators
- 10 have made attempts to identify the NANB agent by ultrastructural changes in hepatocytes in infected individuals. H. Gupta, Liver 8:111-115 (1988); D.W. Bradley J. Virol. Methods 10:307-319 (1985). Similar ultrastructural changes in hepatocytes as well as PCR amplified HCV RNA sequences have been detected in NANBH patients as well as in chimps experimentally infected with infectious HCV
- 15 plasma. T. Shimizu et al., Proc. Natl. Acad. Sci. 87:6441-6444 (1990).

- Considerable serological evidence has been found to implicate HCV as the etiological agent for post-transfusion NANBH. H. Alter et al., N. Eng. J. Med. 321:1494-1500 (1989); Establen et al., The Lancet Aug. 5:294-296 (1989); C. Van Der Poel et al., The Lancet Aug. 5:297-298 (1989); G. Sbolli, J. Med. Virol. 30:230-232 (1990); M. Makris et al., The Lancet 335:1117-1119 (1990).
- 20 Although the detection of HCV antibodies eliminates 70 to 80% of NANBH infected blood from the blood supply system, the antibodies apparently are readily detected during the chronic state of the disease, while only 60% of the samples from the acute NANBH stage are HCV antibody positive. H. Alter et al., New Eng. J. Med. 321:1494-1500 (1989). The prolonged interval between exposure to HCV and antibody detection, and the lack of adequate information regarding the profile of immune response to various structural and non-structural proteins raises questions regarding the infectious state of the patient in the latent and antibody negative phase during NANBH infection.
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- 30 Since discovery of the putative HCV etiological agent as discussed supra, investigators have attempted to express the putative HCV proteins in human expression systems and also to isolate the virus. To date, no report has been published in which HCV has been expressed efficiently in mammalian expression systems, and the virus has not been propagated in tissue culture systems.

- 35 Therefore, there is a need for the development of assay reagents and assay systems to identify acute infection and viremia which may be present, and not currently detected by commercially-available assays. These tools are needed to

help distinguish between acute and persistent, on-going and/or chronic infection from those likely to be resolved, and to define the prognostic course of NANBH infection, in order to develop preventive and/or therapeutic strategies. Also, the expression systems that allow for secretion of these glycosylated antigens would be helpful to purify and manufacture diagnostic and therapeutic reagents.

Summary Of The Invention

This invention provides novel mammalian expression systems that are capable of generating high levels of expressed proteins of HCV. In particular, full-length structural fragments of HCV are expressed as a fusion with the Amyloid Precursor Protein (APP) or Human Growth Hormone (HGH) secretion signal. These unique expression systems allow for the production of high levels of HCV proteins, contributing to the proper processing, glycosylation and folding of the viral protein(s) in the system. In particular, the present invention provides the plasmids pHCV-162, pHCV-167, pHCV-168, pHCV-169 and pHCV-170. The APP-HCV-E2 fusion proteins expressed by mammalian expression vectors pHCV-162 and pHCV-167 also are included. Further, HGH-HCV-E2 fusion proteins expressed by a mammalian expression vectors pHCV-168, pHCV-169 and pHCV-170 are provided.

The present invention also provides a method for detecting HCV antigen or antibody in a test sample suspected of containing HCV antigen or antibody, wherein the improvement comprises contacting the test sample with a glycosylated HCV antigen produced in a mammalian expression system. Also provided is a method for detecting HCV antigen or antibody in a test sample suspected of containing HCV antigen or antibody, wherein the improvement comprises contacting the test sample with an antibody produced by using a glycosylated HCV antigen produced in a mammalian expression system. The antibody can be monoclonal or polyclonal.

The present invention further provides a test kit for detecting the presence of HCV antigen or HCV antibody in a test sample suspected of containing said HCV antigen or antibody, comprising a container containing a glycosylated HCV antigen produced in a mammalian expression system. The test kit also can include an antibody produced by using a glycosylated HCV antigen produced in a mammalian expression system. Another test kit provided by the present invention comprises a container containing an antibody produced by using a glycosylated HCV antigen produced in a mammalian expression system. The antibody provided by the test kits can be monoclonal or polyclonal.

Brief Description of the Drawings

Figure 1 presents a schematic representation of the strategy employed to generate and assemble HCV genomic clones.

Figure 2 presents a schematic representation of the location and amino acid composition of the APP-HCV-E2 fusion proteins expressed by the mammalian expression vectors pHCV-162 and pHCV-167.

Figure 3 presents a schematic representation of the mammalian expression vector pRC/CMV.

Figure 4 presents the RIPA results obtained for the APP-HCV-E2 fusion protein expressed by pHCV-162 in HEK-293 cells using HCV antibody positive human sera.

Figure 5 presents the RIPA results obtained for the APP-HCV-E2 fusion protein expressed by pHCV-162 in HEK-293 cells using rabbit polyclonal sera directed against synthetic peptides.

Figure 6 presents the RIPA results obtained for the APP-HCV-E2 fusion protein expressed by pHCV-167 in HEK-293 cells using HCV antibody positive human sera.

Figure 7 presents the Endoglycosidase-H digestion of the immunoprecipitated APP-HCV-E2 fusion proteins expressed by pHCV-162 and pHCV-167 in HEK-293 cells.

Figure 8 presents the RIPA results obtained when American HCV antibody positive sera were screened against the APP-HCV-E2 fusion protein expressed by pHCV-162 in HEK-293 cells.

Figure 9 presents the RIPA results obtained when the sera from Japanese volunteer blood donors were screened against the APP-HCV-E2 fusion protein expressed by pHCV-162 in HEK-293 cells.

Figure 10 presents the RIPA results obtained when the sera from Japanese volunteer blood donors were screened against the APP-HCV-E2 fusion protein expressed by pHCV-162 in HEK-293 cells.

Figure 11 presents a schematic representation of the mammalian expression vector pCDNA-I.

Figure 12 presents a schematic representation of the location and amino acid composition of the HGH-HCV-E1 fusion protein expressed by the mammalian expression vector pHCV-168.

Figure 13 presents a schematic representation of the location and amino acid composition of the HGH-HCV-E2 fusion proteins expressed by the mammalian expression vectors pHCV-169 and pHCV-170.

Figure 14 presents the RIPA results obtained when HCV E2 antibody positive sera were screened against the HGH-HCV-E1 fusion protein expressed by pHCV-168 in HEK-293 cells.

Figure 15 presents the RIPA results obtained when HCV E2 antibody positive sera were screened against the HGH-HCV-E2 fusion proteins expressed by pHCV-169 and pHCV-170 in HEK-293 cells.

Detailed Description of the Invention

The present invention provides full-length genomic clones useful in a variety of aspects. Such full-length genomic clones can allow culture of the HCV virus which in turn is useful for a variety of purposes. Successful culture of the HCV virus can allow for the development of viral replication inhibitors, viral proteins for diagnostic applications, viral proteins for therapeutics, and specifically structural viral antigens, including, for example, HCV putative envelope, HCV putative E1 and HCV putative E2 fragments.

Cell lines which can be used for viral replication are numerous, and include (but are not limited to), for example, primary hepatocytes, permanent or semi-permanent hepatocytes, cultures transfected with transforming viruses or transforming genes. Especially useful cell lines could include, for example, permanent hepatocyte cultures that continuously express any of several heterologous RNA polymerase genes to amplify HCV RNA sequences under the control of these specific RNA polymerase sequences.

Sources of HCV viral sequences encoding structural antigens include putative core, putative E1 and putative E2 fragments. Expression can be performed in both prokaryotic and eukaryotic systems. The expression of HCV proteins in mammalian expression systems allows for glycosylated proteins such as the E1 and E2 proteins, to be produced. These glycosylated proteins have diagnostic utility in a variety of aspects, including, for example, assay systems for screening and prognostic applications. The mammalian expression of HCV viral proteins allows for inhibitor studies including elucidation of specific viral attachment sites or sequences and/or viral receptors on susceptible cell types, for example, liver cells and the like.

The procurement of specific expression clones developed as described herein in mammalian expression systems provides antigens for diagnostic assays which can determine the stage of HCV infection, such as, for example, acute versus on-going or persistent infections, and/or recent infection versus past exposure. These specific expression clones also provide prognostic markers for resolution of disease such as to distinguish resolution of disease from chronic hepatitis caused by HCV. It is

contemplated that earlier s roconversion to glycosylated structural antigens possibly may be detected by using proteins produced in these mammalian expression systems. Antibodies, both monoclonal and polyclonal, also may be produced from the proteins derived from these mammalian expression systems which then in turn may
5 be used for diagnostic, prognostic and therapeutic applications. Also, reagents produced from these novel expression systems described herein may be useful in the characterization and or isolation of other infectious agents.

Proteins produced from these mammalian expression systems, as well as reagents produced from these proteins, can be placed into appropriate container and
10 packaged as test kits for convenience in performing assays. Other aspects of the present invention include a polypeptide comprising an HCV epitope attached to a solid phase and an antibody to an HCV epitope attached to a solid phase. Also included are methods for producing a polypeptide containing an HCV epitope comprising incubating host cells transformed with a mammalian expression vector containing a
15 sequence encoding a polypeptide containing an HCV epitope under conditions which allow expression of the polypeptide, and a polypeptide containing an HCV epitope produced by this method.

The present invention provides assays which utilize the recombinant or synthetic polypeptides provided by the invention, as well as the antibodies described
20 herein in various formats, any of which may employ a signal generating compound in the assay. Assays which do not utilize signal generating compounds to provide a means of detection also are provided. All of the assays described generally detect either antigen or antibody, or both, and include contacting a test sample with at least one reagent provided herein to form at least one antigen/antibody complex and
25 detecting the presence of the complex. These assays are described in detail herein.

Vaccines for treatment of HCV infection comprising an immunogenic peptide obtained from a mammalian expression system containing an HCV epitope, or an inactivated preparation of HCV, or an attenuated preparation of HCV also are included in the present invention. Also included in the present invention is a method
30 for producing antibodies to HCV comprising administering to an individual an isolated immunogenic polypeptide containing an HCV epitope in an amount sufficient to produce an immune response in the inoculated individual.

Also provided by the present invention is a tissue culture grown cell infected with HCV.

35 The term "antibody containing body component"(or test sample) refers to a component of an individual's body which is the source of the antibodies of interest. These components are well known in the art. These samples include biological

samples which can be tested by the methods of the present invention described herein and include human and animal body fluids such as whole blood, serum, plasma, cerebrospinal fluid, urine, lymph fluids, and various external sections of the respiratory, intestinal and genitourinary tracts, tears, saliva, milk, white blood cells, myelomas and the like, biological fluids such as cell culture supernatants, fixed tissue specimens and fixed cell specimens.

After preparing recombinant proteins, as described by the present invention, the recombinant proteins can be used to develop unique assays as described herein to detect either the presence of antigen or antibody to HCV. These compositions also can be used to develop monoclonal and/or polyclonal antibodies with a specific recombinant protein which specifically binds to the immunological epitope of HCV which is desired by the routineer. Also, it is contemplated that at least one recombinant protein of the invention can be used to develop vaccines by following methods known in the art.

It is contemplated that the reagent employed for the assay can be provided in the form of a kit with one or more containers such as vials or bottles, with each container containing a separate reagent such as a monoclonal antibody, or a cocktail of monoclonal antibodies, or a polypeptide (either recombinant or synthetic) employed in the assay.

"Solid phases" ("solid supports") are known to those in the art and include the walls of wells of a reaction tray, test tubes, polystyrene beads, magnetic beads, nitrocellulose strips, membranes, microparticles such as latex particles, and others. The "solid phase" is not critical and can be selected by one skilled in the art. Thus, latex particles, microparticles, magnetic or non-magnetic beads, membranes, plastic tubes, walls of microtiter wells, glass or silicon chips and sheep red blood cells are all suitable examples. Suitable methods for immobilizing peptides on solid phases include ionic, hydrophobic, covalent interactions and the like. A "solid phase", as used herein, refers to any material which is insoluble, or can be made insoluble by a subsequent reaction. The solid phase can be chosen for its intrinsic ability to attract and immobilize the capture reagent. Alternatively, the solid phase can retain an additional receptor which has the ability to attract and immobilize the capture reagent. The additional receptor can include a charged substance that is oppositely charged with respect to the capture reagent itself or to a charged substance conjugated to the capture reagent. As yet another alternative, the receptor molecule can be any specific binding molecule which is immobilized upon (attached to) the solid phase and which has the ability to immobilize the capture reagent through a specific binding reaction. The receptor molecule enables

the indirect binding of the capture reagent to a solid phase material before the performance of the assay or during the performance of the assay. The solid phase thus can be a plastic, derivatized plastic, magnetic or non-magnetic metal, glass or silicon surface of a test tube, microtiter well, sheet, bead, microparticle, chip, and other configurations known to those of ordinary skill in the art.

It is contemplated and within the scope of the invention that the solid phase also can comprise any suitable porous material with sufficient porosity to allow access by detection antibodies and a suitable surface affinity to bind antigens. Microporous structures are generally preferred, but materials with gel structure in the hydrated state may be used as well. Such useful solid supports include:

natural polymeric carbohydrates and their synthetically modified, cross-linked or substituted derivatives, such as agar, agarose, cross-linked alginic acid, substituted and cross-linked guar gums, cellulose esters, especially with nitric acid and carboxylic acids, mixed cellulose esters, and cellulose ethers; natural polymers containing nitrogen, such as proteins and derivatives, including cross-linked or modified gelatins; natural hydrocarbon polymers, such as latex and rubber; synthetic polymers which may be prepared with suitably porous structures, such as vinyl polymers, including polyethylene, polypropylene, polystyrene, polyvinylchloride, polyvinylacetate and its partially hydrolyzed derivatives, polyacrylamides, polymethacrylates, copolymers and terpolymers of the above polycondensates, such as polyesters, polyamides, and other polymers, such as polyurethanes or polyepoxides; porous inorganic materials such as sulfates or carbonates of alkaline earth metals and magnesium, including barium sulfate, calcium sulfate, calcium carbonate, silicates of alkali and alkaline earth metals, aluminum and magnesium; and aluminum or silicon oxides or hydrates, such as clays, alumina, talc, kaolin, zeolite, silica gel, or glass (these materials may be used as filters with the above polymeric materials); and mixtures or copolymers of the above classes, such as graft copolymers obtained by initializing polymerization of synthetic polymers on a pre-existing natural polymer. All of these materials may be used in suitable shapes, such as films, sheets, or plates, or they may be coated onto or bonded or laminated to appropriate inert carriers, such as paper, glass, plastic films, or fabrics.

The porous structure of nitrocellulose has excellent absorption and adsorption qualities for a wide variety of reagents including monoclonal antibodies. Nylon also possesses similar characteristics and also is suitable. It is contemplated that such porous solid supports described hereinabove are preferably in the form of sheets of thickness from about 0.01 to 0.5 mm, preferably about 0.1 mm. The pore

size may vary within wide limits, and is preferably from about 0.025 to 15 microns, especially from about 0.15 to 15 microns. The surfaces of such supports may be activated by chemical processes which cause covalent linkage of the antigen or antibody to the support. The irreversible binding of the antigen or antibody is
5 obtained, however, in general, by adsorption on the porous material by poorly understood hydrophobic forces. Suitable solid supports also are described in U.S. Patent Application Serial No. 227,272.

The "indicator reagent" comprises a "signal generating compound" (label) which is capable of generating a measurable signal detectable by external means
10 conjugated (attached) to a specific binding member for HCV. "Specific binding member" as used herein means a member of a specific binding pair. That is, two different molecules where one of the molecules through chemical or physical means specifically binds to the second molecule. In addition to being an antibody member of a specific binding pair for HCV, the indicator reagent also can be a member of any
15 specific binding pair, including either hapten-anti-hapten systems such as biotin or anti-biotin, avidin or biotin, a carbohydrate or a lectin, a complementary nucleotide sequence, an effector or a receptor molecule, an enzyme cofactor and an enzyme, an enzyme inhibitor or an enzyme, and the like. An immunoreactive specific binding member can be an antibody, an antigen, or an antibody/antigen
20 complex that is capable of binding either to HCV as in a sandwich assay, to the capture reagent as in a competitive assay, or to the ancillary specific binding member as in an indirect assay.

The various "signal generating compounds" (labels) contemplated include chromogens, catalysts such as enzymes, luminescent compounds such as fluorescein
25 and rhodamine, chemiluminescent compounds, radioactive elements, and direct visual labels. Examples of enzymes include alkaline phosphatase, horseradish peroxidase, beta-galactosidase, and the like. The selection of a particular label is not critical, but it will be capable of producing a signal either by itself or in conjunction with one or more additional substances.

30 The various "signal generating compounds" (labels) contemplated include chromogens, catalysts such as enzymes, luminescent compounds such as fluorescein and rhodamine, chemiluminescent compounds such as acridinium, phenanthridinium and dioxetane compounds, radioactive elements, and direct visual
35 labels. Examples of enzymes include alkaline phosphatase, horseradish peroxidase, beta-galactosidase, and the like. The selection of a particular label is not critical, but it will be capable of producing a signal either by itself or in conjunction with one or more additional substances.

Other embodiments which utilize various other solid phases also are contemplated and are within the scope of this invention. For example, ion capture procedures for immobilizing an immobilizable reaction complex with a negatively charged polymer, described in co-pending U. S. Patent Application Serial No. 150,278 corresponding to EP publication 0326100, and U. S. Patent Application Serial No. 375,029 (EP publication no. 0406473) both of which enjoy common ownership and are incorporated herein by reference, can be employed according to the present invention to effect a fast solution-phase immunochemical reaction. An immobilizable immune complex is separated from the rest of the reaction mixture by ionic interactions between the negatively charged poly-anion/immune complex and the previously treated, positively charged porous matrix and detected by using various signal generating systems previously described, including those described in chemiluminescent signal measurements as described in co-pending U.S. Patent Application Serial No. 921,979 corresponding to EPO Publication No. 0 273,115, which enjoys common ownership and which is incorporated herein by reference.

Also, the methods of the present invention can be adapted for use in systems which utilize microparticle technology including in automated and semi-automated systems wherein the solid phase comprises a microparticle. Such systems include those described in pending U. S. Patent Applications 425,651 and 425,643, which correspond to published EPO applications Nos. EP 0 425 633 and EP 0 424 634, respectively, which are incorporated herein by reference.

The use of scanning probe microscopy (SPM) for immunoassays also is a technology to which the monoclonal antibodies of the present invention are easily adaptable. In scanning probe microscopy, in particular in atomic force microscopy, the capture phase, for example, at least one of the monoclonal antibodies of the invention, is adhered to a solid phase and a scanning probe microscope is utilized to detect antigen/antibody complexes which may be present on the surface of the solid phase. The use of scanning tunnelling microscopy eliminates the need for labels which normally must be utilized in many immunoassay systems to detect antigen/antibody complexes. Such a system is described in pending U. S. patent application Serial No. 662,147, which enjoys common ownership and is incorporated herein by reference.

The use of SPM to monitor specific binding reactions can occur in many ways. In one embodiment, one member of a specific binding partner (analyte specific substance which is the monoclonal antibody of the invention) is attached to a surface suitable for scanning. The attachment of the analyte specific substance may be by adsorption to a test piece which comprises a solid phase of a plastic or

metal surface, following methods known to those of ordinary skill in the art. Or, covalent attachment of a specific binding partner (analyte specific substance) to a test piece which test piece comprises a solid phase of derivatized plastic, metal, silicon, or glass may be utilized. Covalent attachment methods are known to those skilled in the art and include a variety of means to irreversibly link specific binding partners to the test piece. If the test piece is silicon or glass, the surface must be activated prior to attaching the specific binding partner. Activated silane compounds such as triethoxy amino propyl silane (available from Sigma Chemical Co., St. Louis, MO), triethoxy vinyl silane (Aldrich Chemical Co., Milwaukee, WI), and (3-mercaptopropyl)-trimethoxy silane (Sigma Chemical Co., St. Louis, MO) can be used to introduce reactive groups such as amino-, vinyl, and thiol, respectively. Such activated surfaces can be used to link the binding partner directly (in the cases of amino or thiol) or the activated surface can be further reacted with linkers such as glutaraldehyde, bis (succinimidyl) suberate, SPPD 9 succinimidyl 3-[2-pyridyldithio] propionate), SMCC (succinimidyl-4-[N-maleimidomethyl] cyclohexane-1-carboxylate), SIAB (succinimidyl [4-iodoacetyl] aminobenzoate), and SMPB (succinimidyl 4-[1-maleimidophenyl] butyrate) to separate the binding partner from the surface. The vinyl group can be oxidized to provide a means for covalent attachment. It also can be used as an anchor for the polymerization of various polymers such as poly acrylic acid, which can provide multiple attachment points for specific binding partners. The amino surface can be reacted with oxidized dextrans of various molecular weights to provide hydrophilic linkers of different size and capacity. Examples of oxidizable dextrans include Dextran T-40 (molecular weight 40,000 daltons), Dextran T-110 (molecular weight 110,000 daltons), Dextran T-500 (molecular weight 500,000 daltons), Dextran T-2M (molecular weight 2,000,000 daltons) (all of which are available from Pharmacia, LOCATION), or Ficoll (molecular weight 70,000 daltons (available from Sigma Chemical Co., St. Louis, MO). Also, polyelectrolyte interactions may be used to immobilize a specific binding partner on a surface of a test piece by using techniques and chemistries described by pending U. S. Patent applications Serial No. 150,278, filed January 29, 1988, and Serial No. 375,029, filed July 7, 1989, each of which enjoys common ownership and each of which is incorporated herein by reference. The preferred method of attachment is by covalent means. Following attachment of a specific binding member, the surface may be further treated with materials such as serum, proteins, or other blocking agents to minimize non-specific binding. The surface also may be scanned either at the site of manufacture or point of use to verify its suitability for assay

purposes. The scanning process is not anticipated to alter the specific binding properties of the test piece.

Various other assay formats may be used, including "sandwich" immunoassays and competitive probe assays. For example, the monoclonal antibodies produced from the proteins of the present invention can be employed in various assay systems to determine the presence, if any, of HCV proteins in a test sample. Fragments of these monoclonal antibodies provided also may be used. For example, in a first assay format, a polyclonal or monoclonal anti-HCV antibody or fragment thereof, or a combination of these antibodies, which has been coated on a solid phase, is contacted with a test sample which may contain HCV proteins, to form a mixture. This mixture is incubated for a time and under conditions sufficient to form antigen/antibody complexes. Then, an indicator reagent comprising a monoclonal or a polyclonal antibody or a fragment thereof, which specifically binds to the HCV fragment, or a combination of these antibodies, to which a signal generating compound has been attached, is contacted with the antigen/antibody complexes to form a second mixture. This second mixture then is incubated for a time and under conditions sufficient to form antibody/antigen/antibody complexes. The presence of HCV antigen present in the test sample and captured on the solid phase, if any, is determined by detecting the measurable signal generated by the signal generating compound. The amount of HCV antigen present in the test sample is proportional to the signal generated.

Alternatively, a polyclonal or monoclonal anti-HCV antibody or fragment thereof, or a combination of these antibodies which is bound to a solid support, the test sample and an indicator reagent comprising a monoclonal or polyclonal antibody or fragments thereof, which specifically binds to HCV antigen, or a combination of these antibodies to which a signal generating compound is attached, are contacted to form a mixture. This mixture is incubated for a time and under conditions sufficient to form antibody/antigen/antibody complexes. The presence, if any, of HCV proteins present in the test sample and captured on the solid phase is determined by detecting the measurable signal generated by the signal generating compound. The amount of HCV proteins present in the test sample is proportional to the signal generated.

In another alternate assay format, one or a combination of one or more monoclonal antibodies of the invention can be employed as a competitive probe for the detection of antibodies to HCV protein. For example, HCV proteins, either alone or in combination, can be coated on a solid phase. A test sample suspected of containing antibody to HCV antigen then is incubated with an indicator reagent

comprising a signal generating compound and at least one monoclonal antibody of the invention for a time and under conditions sufficient to form antigen/antibody complexes of either the test sample and indicator reagent to the solid phase or the indicator reagent to the solid phase. The reduction in binding of the monoclonal antibody to the solid phase can be quantitatively measured. A measurable reduction in the signal compared to the signal generated from a confirmed negative NANB hepatitis test sample indicates the presence of anti-HCV antibody in the test sample.

In yet another detection method, each of the monoclonal antibodies of the present invention can be employed in the detection of HCV antigens in fixed tissue sections, as well as fixed cells by immunohistochemical analysis.

In addition, these monoclonal antibodies can be bound to matrices similar to CNBr-activated Sepharose and used for the affinity purification of specific HCV proteins from cell cultures, or biological tissues such as blood and liver.

The monoclonal antibodies of the invention can also be used for the generation of chimeric antibodies for therapeutic use, or other similar applications.

The monoclonal antibodies or fragments thereof can be provided individually to detect HCV antigens. Combinations of the monoclonal antibodies (and fragments thereof) provided herein also may be used together as components in a mixture or "cocktail" of at least one anti-HCV antibody of the invention with antibodies to other HCV regions, each having different binding specificities. Thus, this cocktail can include the monoclonal antibodies of the invention which are directed to HCV proteins and other monoclonal antibodies to other antigenic determinants of the HCV genome.

The polyclonal antibody or fragment thereof which can be used in the assay formats should specifically bind to a specific HCV region or other HCV proteins used in the assay. The polyclonal antibody used preferably is of mammalian origin; human, goat, rabbit or sheep anti-HCV polyclonal antibody can be used. Most preferably, the polyclonal antibody is rabbit polyclonal anti-HCV antibody. The polyclonal antibodies used in the assays can be used either alone or as a cocktail of polyclonal antibodies. Since the cocktails used in the assay formats are comprised of either monoclonal antibodies or polyclonal antibodies having different HCV specificity, they would be useful for diagnosis, evaluation and prognosis of HCV infection, as well as for studying HCV protein differentiation and specificity.

In another assay format, the presence of antibody and/or antigen to HCV can be detected in a simultaneous assay, as follows. A test sample is simultaneously contacted with a capture reagent of a first analyte, wherein said capture reagent

comprises a first binding member specific for a first analyte attached to a solid phase and a capture reagent for a second analyte, wherein said capture reagent comprises a first binding member for a second analyte attached to a second solid phase, to thereby form a mixture. This mixture is incubated for a time and under conditions sufficient to form capture reagent/first analyte and capture reagent/second analyte complexes. These so-formed complexes then are contacted with an indicator reagent comprising a member of a binding pair specific for the first analyte labelled with a signal generating compound and an indicator reagent comprising a member of a binding pair specific for the second analyte labelled with a signal generating compound to form a second mixture. This second mixture is incubated for a time and under conditions sufficient to form capture reagent/first analyte/indicator reagent complexes and capture reagent/second analyte/indicator reagent complexes. The presence of one or more analytes is determined by detecting a signal generated in connection with the complexes formed on either or both solid phases as an indication of the presence of one or more analytes in the test sample. In this assay format, proteins derived from human expression systems may be utilized as well as monoclonal antibodies produced from the proteins derived from the mammalian expression systems as disclosed herein. Such assay systems are described in greater detail in pending U.S. Patent Application Serial No. 07/574,821 entitled Simultaneous Assay for Detecting One Or More Analytes, filed August 29, 1990, which enjoys common ownership and is incorporated herein by reference.

In yet other assay formats, recombinant proteins may be utilized to detect the presence of anti-HCV in test samples. For example, a test sample is incubated with a solid phase to which at least one recombinant protein has been attached. These are reacted for a time and under conditions sufficient to form antigen/antibody complexes. Following incubation, the antigen/antibody complex is detected. Indicator reagents may be used to facilitate detection, depending upon the assay system chosen. In another assay format, a test sample is contacted with a solid phase to which a recombinant protein produced as described herein is attached and also is contacted with a monoclonal or polyclonal antibody specific for the protein, which preferably has been labelled with an indicator reagent. After incubation for a time and under conditions sufficient for antibody/antigen complexes to form, the solid phase is separated from the free phase, and the label is detected in either the solid or free phase as an indication of the presence of HCV antibody. Other assay formats utilizing the proteins of the present invention are contemplated. These include contacting a test sample with a solid phase to which at

least one recombinant protein produced in the mammalian expression system has been attached, incubating the solid phase and test sample for a time and under conditions sufficient to form antigen/antibody complexes, and then contacting the solid phase with a labelled recombinant antigen. Assays such as this and others are described in pending U.S. Patent Application Serial No. 07/787,710, which enjoys common ownership and is incorporated herein by reference.

While the present invention discloses the preference for the use of solid phases, it is contemplated that the proteins of the present invention can be utilized in non-solid phase assay systems. These assay systems are known to those skilled in the art, and are considered to be within the scope of the present invention.

The present invention will now be described by way of examples, which are meant to illustrate, but not to limit, the spirit and scope of the invention.

EXAMPLES

Example 1: Generation of HCV Genomic Clones

RNA isolated from the serum or plasma of a chimpanzee (designated as "CO") experimentally infected with HCV, or an HCV seropositive human patient (designated as "LG") was transcribed to cDNA using reverse transcriptase employing either random hexamer primers or specific anti-sense primers derived from the prototype HCV-1 sequence. The sequence has been reported by Choo et al. (Choo et al., Proc. Nat'l. Acad. Sci. USA 88:2451-2455 [1991], and is available through GenBank data base, Accession No. M62321). This cDNA then was amplified using PCR and AmpliTaq® DNA polymerase (available in the Gene Amp Kit® from Perkin Elmer Cetus, Norwalk, Connecticut 06859) employing either a second sense primer located approximately 1000-2000 nucleotides upstream of the specific antisense primer or a pair of sense and antisense primers flanking a 1000-2000 nucleotide fragment of HCV. After 25 to 35 cycles of amplification following standard procedures known in the art, an aliquot of this reaction mixture was subjected to nested PCR (or "PCR-2"), wherein a pair of sense and antisense primers located internal to the original pair of PCR primers was employed to further amplify HCV gene segments in quantities sufficient for analysis and subcloning, utilizing endonuclease recognition sequences present in the second set of PCR primers. In this manner, seven adjacent HCV DNA fragments were generated which then could be assembled using the generic cloning strategy presented and described in FIGURE 1. The location of the specific primers used in this manner are presented in Table 1 and are numbered according to the HCV-1 sequence reported by Choo et al (GenBank data base, Accession No. M62321). Prior to

assembly, the DNA sequence of each of the individual fragments was determined and translated into the genomic amino acid sequences presented in SEQUENCE ID. NO. 1 and 2, respectively, for CO and LG, respectively. Comparison of the genomic polypeptide of CO with that of HCV-1 demonstrated 98 amino acid differences.

5 Comparison of the genomic polypeptide of CO with that of LG. demonstrated 150 amino acid differences. Comparison of the genomic polypeptide of LG with that of HCV-1 demonstrated 134 amino acid differences.

Example 2. Expression of the HCV E2 Protein As A Fusion
With The Amyloid Precursor Protein (APP)

10 The HCV E2 protein from CO developed as described in Example 1 was expressed as a fusion with the Amyloid Precursor Protein (APP). APP has been described by Kang et al., Nature 325:733-736 (1987). Briefly, HCV amino acids 384-749 of the CO isolate were used to replace the majority of the APP coding

15 sequence as demonstrated in FIGURE 2. A HindIII-Styl DNA fragment representing the amino-terminal 66 amino acids and a BglII-XbaI fragment representing the carboxyl-terminal 105 amino acids of APP were ligated to a PCR derived HCV fragment from CO representing HCV amino acids 384-749 containing Styl and BglII restriction sites on its 5' and 3' ends, respectively. This APP-HCV-E2 fusion gene

20 cassette then was cloned into the commercially available mammalian expression vector pRC/CMV shown in FIGURE 3, (available from Invitrogen, San Diego, CA) at the unique HindIII and XbaI sites. After transformation into E. coli DH5a, a clone designated pHCV-162 was isolated, which placed the expression of the APP-HCV-E2 fusion gene cassette under control of the strong CMV promotor. The complete

25 nucleotide sequence of the mammalian expression vector pHCV-152 is presented in SEQUENCE ID. NO. 3. Translation of nucleotides 922 through 2535 results in the complete amino acid sequence of the APP-HCV-E2 fusion protein expressed by pHCV-162 as presented in SEQUENCE ID. NO. 4.

A primary Human Embryonic Kidney (HEK) cell line transformed with

30 human adenovirus type 5, designated as HEK-293, was used for all transfections and expression analyses. HEK-293 cells were maintained in Minimum Essential Medium (MEM) which was supplemented with 10% fetal calf serum (FCS), penicillin and streptomycin.

Approximately 20 µg of purified DNA from pHCV-162 was transfected into

35 HEK-293 cells using the modified calcium phosphate protocol as reported by Chen et al., Molecular and Cellular Biology 7(8):2745-2752 (1987). The calcium-phosphate-DNA solution was incubated on the HEK-293 cells for about 15 to 24

hours. The solution was removed, the cells were washed twice with MEM media, and then the cells were incubated in MEM media for an additional 24 to 48 hours. In order to analyze protein expression, the transfected cells were metabolically labelled with 100 μ Ci/ml S-35 methionine and cysteine for 12 to 18 hours. The culture media was removed and stored, and the cells were washed in MEM media and then lysed in phosphate buffered saline (PBS) containing 1% Triton X-100® (available from Sigma Chemical Co., St. Louis, MO), 0.1% sodium dodecyl sulfate (SDS), and 0.5% deoxycholate, designated as PBS-TDS. This cell lysate then was frozen at -70°C for 2 to 24 hours, thawed on ice and then clarified by centrifugation at 50,000 x g force for one hour at 4°C. Standard radio-immunoprecipitation assays (RIPAs) then were conducted on those labelled cell lysates and/or culture medias. Briefly, labelled cell lysates and/or culture medias were incubated with 2 to 5 μ l of specific sera at 4°C for one hour. Protein-A sepharose then was added and the samples were further incubated for one hour at 4°C with agitation. The samples were then centrifuged and the pellets washed several times with PBS-TDS buffer. Proteins recovered by immunoprecipitation were eluted by heating in an electrophoresis sample buffer (50 mM Tris-HCl, pH 6.8, 100 mM dithiothreitol [DTT], 2% SDS, 0.1% bromophenol blue, and 10% glycerol) for five minutes at 95°C. The eluted proteins then were separated by SDS polyacrylamide gels which were subsequently treated with a fluorographic reagent such as Enlightening® (available from NEN [DuPont], Boston, MA), dried under vacuum and exposed to x-ray film at -70°C with intensifying screens. FIGURE 4 presents a RIPA analysis of pHCV-162 transfected HEK cell lysate precipitated with normal human sera (NHS), a monoclonal antibody directed against APP sequences which were replaced in this construct (MAB), and an HCV antibody positive human sera (#25). Also presented in FIGURE 4 is the culture media (supernatant) precipitated with the same HCV antibody positive human sera (#25). From FIGURE 4, it can be discerned that while only low levels of an HCV specific protein of approximately 75K daltons is detected in the culture media of HEK-293 cells transfected with pHCV-162, high levels of intracellular protein expression of the APP-HCV-E2 fusion protein of approximately 70K daltons is evident.

In order to further characterize this APP-HCV-E2 fusion protein, rabbit polyclonal antibody raised against synthetic peptides were used in a similar RIPA, the results of which are illustrated in FIGURE 5. As can be discerned from this Figure, normal rabbit serum (NRS) does not precipitate the 70K dalton protein while rabbit sera raised against HCV amino acids 509-551 (6512), HCV amino

acids 380-436 (6521), and APP amino acids 45-62 (anti- N-terminus) are highly specific for the 70K dalton APP-HCV-E2 fusion protein.

In order to enhance secretion of this APP-HCV-E2 fusion protein, another clone was generated which fused only the amino-terminal 66 amino acids of APP, which contain the putative secretion signal sequences to the HCV-E2 sequences. In addition, a strongly hydrophobic sequence at the carboxyl-terminal end of the HCV-E2 sequence which was identified as a potential transmembrane spanning region was deleted. The resulting clone was designated as pHCV-167 and is schematically illustrated in FIGURE 2. The complete nucleotide sequence of the mammalian expression vector pHCV-167 is presented in SEQUENCE ID. NO. 5. Translation of nucleotides 922 through 2025 results in the complete amino acid sequence of the APP-HCV-E2 fusion protein expressed by pHCV-167 as presented in SEQUENCE ID. NO. 6. Purified DNA of pHCV-167 was transfected into HEK-293 cells and analyzed by RIPA and polyacrylamide SDS gels as described previously herein. FIGURE 6 presents the results in which a normal human serum sample (NHS) failed to recognize the APP-HCV-E2 fusion protein present in either the cell lysate or the cell supernatant of HEK-293 cells transfected with pHCV-167. The positive control HCV serum sample (#25), however, precipitated an approximately 65K dalton APP-HCV-E2 fusion protein present in the cell lysate of HEK-293 cells transfected with pHCV-167. In addition, substantial quantities of secreted APP-HCV-E2 protein of approximately 70K daltons was precipitated from the culture media by serum #25.

Digestion with Endoglycosidase-H (Endo-H) was conducted to ascertain the extent and composition of N-linked glycosylation in the APP-HCV E2 fusion proteins expressed by pHCV-167 and pHCV-162 in HEK-293 cells. Briefly, multiple aliquots of labelled cell lysates from pHCV-162 and pHCV-167 transfected HEK-293 cells were precipitated with human serum #50 which contained antibody to HCV E2 as previously described. The Protein-A sepharose pellet containing the immunoprecipitated protein-antibody complex was then resuspended in buffer (75mM sodium acetate, 0.05% SDS) containing or not containing 0.05 units per ml of Endo-H (Sigma). Digestions were performed at 37°C for 12 to 18 hours and all samples were analyzed by polyacrylamide SDS gels as previously described. FIGURE 7 presents the results of Endo-H digestion. Carbon-14 labelled molecular weight standards (MW) (obtained from Amersham, Arlington Heights, IL) are common on all gels and represent 200K, 92.5K, 69K, 46K, 30K and 14.3K daltons, respectively. Normal human serum (NHS) does not immunoprecipitate the APP-HCV-E2 fusion protein expressed by either pHCV-162 or pHCV-167, while

human serum positive for HCV E2 antibody (#50) readily detects the 72K dalton APP-HCV-E2 fusion protein in pHCV-162 and the 65K dalton APP-HCV E2 fusion protein in pHCV-167. Incubation of these immunoprecipitated proteins in the absence of Endo-H (#50 -Endo-H) does not significantly affect the quantity or mobility of either pHCV-162 or pHCV-167 expressed proteins. Incubation in the presence of Endo-H (#50 +Endo-H), however, drastically reduces the mobility of the proteins expressed by pHCV-162 and pHCV-167, producing a heterogeneous size distribution. The predicted molecular weight of the non-glycosylated polypeptide backbone of pHCV-162 is approximately 59K daltons. Endo-H treatment of pHCV-162 lowers the mobility to a minimum of approximately 44K daltons, indicating that the APP-HCV-E2 fusion protein produced by pHCV-162 is proteolytically cleaved at the carboxyl-terminal end. A size of approximately 44K daltons is consistent with cleavage at or near HCV amino acid 720. Similarly, Endo-H treatment of pHCV-167 lowers the mobility to a minimum of approximately 41K daltons, which compares favorably with the predicted molecular weight of approximately 40K daltons for the intact APP-HCV-E2 fusion protein expressed by pHCV-167.

Example 3 Detection of HCV E2 Antibodies

Radio-immunoprecipitation assay (RIPA) and polyacrylamide SDS gel analysis previously described was used to screen numerous serum samples for the presence of antibody directed against HCV E2 epitopes. HEK-293 cells transfected with pHCV-162 were metabolically labelled and cell lysates prepared as previously described. In addition to RIPA analysis, all serum samples were screened for the presence of antibodies directed against specific HCV recombinant antigens representing distinct areas of the HCV genome using the Abbott Matrix® System. (available from Abbott Laboratories, Abbott Park, IL 60064, U.S. No. Patent 5,075,077). In the Matrix data presented in Tables 2 through 7, C100 yeast represents the NS4 region containing HCV amino acids 1569-1930, C100 E.coli represents HCV amino acids 1676-1930, NS3 represents HCV amino acids 1192-1457, and CORE represents HCV amino acids 1-150.

FIGURE 8 presents a representative RIPA result obtained using pHCV-162 cell lysate to screen HCV antibody positive American blood donors and transfusion recipients. Table 2 summarizes the antibody profile of these various American blood samples, with seven of seventeen (41%) samples demonstrating HCV E2 antibody. Genomic variability in the E2 region has been demonstrated between different HCV isolates, particularly in geographically distinct isolates which may

lead to differences in antibody responses. We therefore screened twenty-six Japanese volunteer blood donors and twenty Spanish hemodialysis patients previously shown to contain HCV antibody for the presence of specific antibody to the APP-HCV E2 fusion protein expressed by pHCV-162. Figures 9 and 10 present the RIPA analysis on twenty-six Japanese volunteer blood donors. Positive control human sera (#50) and molecular weight standards (MW) appear in both figures in which the specific immunoprecipitation of the approximately 72K dalton APP-HCV-E2 fusion protein is demonstrated for several of the serum samples tested. Table 3 presents both the APP-HCV-E2 RIPA and Abbott Matrix® results summarizing the antibody profiles of each of the twenty-six Japanese samples tested. Table 4 presents similar data for the twenty Spanish hemodialysis patients tested. Table 5 summarizes the RIPA results obtained using pHCV-162 to detect HCV E2 specific antibody in these various samples. Eighteen of twenty-six (69%) Japanese volunteers blood donors, fourteen of twenty (70%) Spanish hemodialysis patients, and seven of seventeen (41%) American blood donors or transfusion recipients demonstrated a specific antibody response against the HCV E2 fusion protein. The broad immunoreactivity demonstrated by the APP-HCV-E2 fusion protein expressed by pHCV-162 suggests the recognition of conserved epitopes within HCV E2.

Serial bleeds from five transfusion recipients which seroconverted to HCV antibody were also screened using the APP-HCV-E2 fusion protein expressed by pHCV-162. This analysis was conducted to ascertain the time interval after exposure to HCV at which E2 specific antibodies can be detected. Table 6 presents one such patient (AN) who seroconverted to NS3 at 154 days post transfusion (DPT). Antibodies to HCV E2 were not detected by RIPA until 271 DPT. Table 7 presents another such patient (WA), who seroconverted to CORE somewhere before 76 DPT and was positive for HCV E2 antibodies on the next available bleed date (103 DPT). Table 8 summarizes the serological results obtained from these five transfusion recipients indicating (a) some general antibody profile at seroconversion (AB Status); (b) the days post transfusion at which an ELISA test would most likely detect HCV antibody (2.0 GEN); (c) the samples in which HCV E2 antibody was detected by RIPA (E2 AB Status); and (d) the time interval covered by the bleed dates tested (Samples Tested). The results indicate that antibody to HCV E2, as detected in the RIPA procedure described here, appears after seroconversion to at least one other HCV marker (CORE, NS3, C100, etc.) and is persistent in nature once it appears. In addition, the absence of antibody to the structural gene CORE appears highly correlated with the absence of detectable antibody to E2,

another putative structural antigen. Further work is ongoing to correlate the presence or absence of HCV gene specific antibodies with progression of disease and/or time interval since exposure to HCV viral antigens.

5

Example 4 Expression of HCV E1 and E2 Using
Human Growth Hormone Secretion Signal

HCV DNA fragments representing HCV E1 (HCV amino acids 192 to 384) and HCV E2 (HCV amino acids 384-750 and 384-684) were generated from the CO isolate using PCR as described in Example 2. An Eco RI restriction site was used to
10 attach a synthetic oligonucleotide encoding the Human Growth Hormone (HGH) secretion signal (Blak et al, Oncogene, 3 129-136, 1988) at the 5' end of these HCV sequence. The resulting fragment was then cloned into the commercially available mammalian expression vector pCDNA-I, (available from Invitrogen, San Diego, California) illustrated in FIGURE 11. Upon transformation into E. coli
15 MC1061/P3, the resulting clones place the expression of the cloned sequence under control of the strong CMV promoter. Following the above outlined methods, a clone capable of expressing HCV-E1 (HCV amino acids 192-384) employing the HGH secretion signal at the extreme amino-terminal end was isolated. The clone was designated pHCV-168 and is schematically illustrated in FIGURE 12. Similarly,
20 clones capable of expressing HCV E2 (HCV amino acids 384-750 or 384-684) employing the HGH secretion signal were isolated, designated pHCV-169 and pHVC-170 respectively and illustrated in FIGURE 13. The complete nucleotide sequence of the mammalian expression vectors pHCV-168, pHCV-169, and pHCV-170 are presented in Sequence ID. NO. 7, 9, and 11 respectively. Translation of
25 nucleotides 2227 through 2813 results in the complete amino acid sequence of the HGH-HCV-E1 fusion protein expressed by pHCV-168 as presented in Sequence ID. NO. 8. Translation of nucleotides 2227 through 3426 results in the complete amino acid sequence of the HGH-HCV-E2 fusion protein expressed by pHCV-169 as presented in Sequence ID. NO. 10. Translation of nucleotides 2227 through 3228
30 results in the complete amino acid sequence of the HGH-HCV-E2 fusion protein expressed by pHCV-170 as presented in Sequence ID. NO. 12. Purified DNA from pHCV-168, pHCV-169, and pHCV-170 was transfected into HEK-293 cells which were then metabolically labelled, cell lysates prepared, and RIPA analysis performed as described previously herein. Seven sera samples previously shown to
35 contain antibodies to the APP-HCV-E2 fusion protein expressed by pHCV-162 were screened against the labelled cell lysates of pHCV-168, pHCV-169, and pHCV-170. Figure 14 presents the RIPA analysis for pHCV-168 and demonstrated that five

sera containing HCV E2 antibodies also contain HCV E1 antibodies directed against as approximately 33K dalton HGH-HCV-E1 fusion protein (#25, #50, 121, 503, and 728), while two other sera do not contain those antibodies (476 and 505). Figure 15 presents the RIPA results obtained when the same sera indicated above were screened against the labelled cell lysates of either pHCV-169 or pHCV-170. All seven HCV E1 antibody positive sera detected two protein species of approximately 70K and 75K daltons in cells transfected with pHCV-168. These two different HGH-HCV-E2 protein species could result from incomplete proteolytic cleavage of the HCV E2 sequence at the carboxyl-terminal end (at or near HCV amino acid 720) or from differences in carbohydrate processing between the two species. All seven HCV E2 antibody positive sera detected a single protein species of approximately 62K daltons for the HGH-HCV-E2 fusion protein expressed by pHCV-170. Table 9 summarizes the serological profile of six of the seven HCV E2 antibody positive sera screened against the HGH-HCV-E1 fusion protein expressed by pHCV-170. Further work is ongoing to correlate the presence or absence of HCV gene specific antibodies with progression of disease and/or time interval since exposure to HCV viral antigens.

Clones pHCV-167 and pHCV-162 have been deposited at the American Type Culture Collection, 12301 Parklawn Drive, Rockville, Maryland, 20852, as of January 17, 1992 under the terms of the Budapest Treaty, and accorded the following ATCC Designation Numbers: Clone pHCV-167 was accorded ATCC deposit number 68893 and clone pHCV-162 was accorded ATCC deposit number 68894. Clones pHCV-168, pHCV-169 and pHCV-170 have been deposited at the American Type Culture Collection, 12301 Parklawn Drive, Rockville, Maryland, 20852, as of January 26, 1993 under the terms of the Budapest Treaty, and accorded the following ATCC Designation Numbers: Clone pHCV-168 was accorded ATCC deposit number 69228, clone pHCV-169 was accorded ATCC deposit number 69229 and clone pHCV-170 was accorded ATCC deposit number 69230. The designated deposits will be maintained for a period of thirty (30) years from the date of deposit, or for five (5) years after the last request for the deposit, or for the enforceable life of the U.S. patent, whichever is longer. These deposits and other deposited materials mentioned herein are intended for convenience only, and are not required to practice the invention in view of the descriptions herein. The HCV cDNA sequences in all of the deposited materials are incorporated herein by reference.

Other variations of applications of the use of the proteins and mammalian expression systems provided herein will be apparent to those skilled in the art.

Accordingly, the invention is intended to be limited only in accordance with the appended claims.

TABLE 1

5

FRAGMENT	PCR-1 PRIMERS		PCR-2 PRIMERS	
	SENSE	ANTISENSE	SENSE	ANTISENSE
1	1-17	1376-1400	14-31	1344-1364
2	1320-1344	2332-2357	1357-1377	2309-2327
3	2288-2312	3245-3269	2322-2337	3224-3242
4	3178-3195	5303-5321	3232-3252	5266-5289
5	5229-5249	6977-6996	5273-5292	6940-6962
6	6907-6925	8221-8240	6934-6954	8193-8216
7	8175-8194	9385-9401	8199-8225	9363-9387

TABLE 2

10

AMERICAN HCV POSITIVE SERA

SAMPLE	C100 YEAST S/CO	C100 E.COLT S/CO	NS3 S/CO	COPE S/CO	E2 RIPA
22	0.31	1.09	1.72	284.36	+
32	0.02	0.10	7.95	331.67	-
35	0.43	0.68	54.61	2.81	-
37	136.24	144.29	104.13	245.38	+
50	101.04	133.69	163.65	263.72	+
108	39.07	34.55	108.79	260.47	-
121	1.28	4.77	172.65	291.82	+
128	0.06	0.06	0.87	298.49	-
129	0.00	0.02	107.11	0.00	-
142	8.45	8.88	73.93	2.32	-
156	0.45	0.14	0.67	161.84	-
163	1.99	3.26	11.32	24.36	-
MI	89.9	118.1	242.6	120.4	-
KE	167.2	250.9	0.8	0.3	-
WA	164.4	203.3	223.9	160.9	+
PA	50.6	78.8	103.8	78.0	+
AN	224.8	287.8	509.9	198.8	+

TABLE 3
JAPANESE HCV POSITIVE POSITIVE BLOOD DONORS

5

SAMPLE	C100 YEAST S/CO	C100 ECOLT S/CO	NS3 S/CO	COPE S/CO	E2 RIPA
410	86.33	93.59	9.68	257.82	+
435	0.18	0.18	0.69	39.25	+
441	0.20	0.09	0.17	6.51	-
476	0.37	1.29	144.66	302.35	+
496	39.06	37.95	2.78	319.99	-
560	1.08	0.68	3.28	26.59	-
589	0.06	1.28	117.82	224.23	+
620	0.17	1.37	163.41	256.64	+
622	123.46	162.54	154.67	243.44	+
623	23.46	26.55	143.72	277.24	+
633	0.01	0.43	161.84	264.02	+
639	1.40	2.23	12.15	289.80	+
641	0.01	0.08	8.65	275.00	+
648	-0.00	0.03	0.79	282.64	+
649	97.00	127.36	147.46	194.73	+
657	4.12	6.33	141.04	256.57	+
666	0.14	0.24	5.90	60.82	-
673	72.64	90.11	45.31	317.66	+
677	0.05	0.23	2.55	99.67	-
694	86.72	87.18	45.43	248.80	+
696	0.02	-0.02	0.26	12.55	-
706	17.02	12.96	153.77	266.87	+
717	0.04	0.02	0.15	10.46	-
728	-0.01	0.26	90.37	246.30	+
740	0.02	0.10	0.25	46.27	-
743	1.95	1.56	133.23	254.25	+

TABLE 4
SPANISH HEMODIALYSIS PATIENTS

5	SAMPLE	C100 YEAST S/CO	C100 ECOLT S/CO	NS3 S/CO	COORE S/CO	E2 RIPA
	1	0.0	0.3	188.6	-0.0	-
	2	129.3	142.8	165.4	201.0	+
	3	113.7	128.5	154.5	283.3	+
	5	130.6	143.8	133.4	186.1	+
	6	56.2	63.4	93.6	32.0	+
	7	0.0	0.2	72.1	211.5	+
	8	156.7	171.9	155.1	227.0	+
	9	65.3	78.9	76.1	102.6	+
	10	136.7	149.3	129.4	190.2	+
	11	0.0	0.7	155.7	272.4	+
	12	1.0	1.9	143.6	210.6	+
	13	0.0	0.3	111.2	91.1	-
	14	1.1	3.1	94.7	214.8	-
	15	45.9	66.1	106.3	168.2	+
	16	36.3	68.8	149.3	0.1	-
	17	121.0	129.9	113.4	227.8	+
	18	64.8	99.7	138.9	0.2	-
	19	25.6	34.1	157.4	254.9	+
	20	104.9	125.1	126.8	218.3	+
	21	48.1	68.5	0.8	49.4	-

TABLE 5
ANTIBODY RESPONSE TO HCV PROTEINS

10		C100 YEAST S/CO	C100 E. COLI S/CO	NS3 S/CO	COORE S/CO	E2 RIPA
	AMERICAN BLOOD DONORS	11/17	12/17	14/17	15/17	7/17
	SPANISH HEMODIALYSIS PATIENTS	16/20	16/20	19/20	17/20	14/20
	JAPANESE BLOOD DONORS	12/26	14/26	20/26	26/26	18/26

TABLE 6
HUMAN TRANSFUSION RECIPIENT (AN)

5	DAYS POST TRANS	C100 YEAST S/CO	C100 E. COLI S/CO	NS3 S/CO	COORE S/CO	E2 RIPA
	29	1.8	1.9	8.9	1.1	-
	57	0.4	0.3	1.2	0.4	-
	88	0.3	0.3	0.4	0.7	-
	116	0.1	0.2	0.5	0.2	-
	154	0.3	0.7	65.3	0.8	-
	179	18.0	21.5	445.6	1.5	-
	271	257.4	347.2	538.0	3.1	+
	376	240.0	382.5	513.5	139.2	+
	742	292.9	283.7	505.3	198.1	+
	1105	282.1	353.9	456.1	202.2	+
	1489	224.8	287.8	509.9	198.8	+

TABLE 7
HUMAN TRANSFUSION RECIPIENT (WA)

10	DAYS POST TRANS	C100 YEAST S/CO	C100 E. COLI S/CO	NS3 S/CO	COORE S/CO	E2 RIPA
	43	0.1	0.6	0.4	1.2	-
	76	0.1	0.1	0.9	72.7	-
	103	0.0	0.6	1.4	184.4	+
	118	3.7	3.7	1.9	208.7	+
	145	83.8	98.9	12.3	178.0	+
	158	142.1	173.8	134.3	185.2	+
	174	164.4	203.3	223.9	160.9	+

TABLE 8
HUMAN TRANSFUSION RECIPIENTS

	<u>AB STATUS</u>	<u>2.0 GEN</u>	<u>E2 AB STATUS</u>	<u>SAMPLES TESTED</u>
MI	STRONG RESPONSE	78 DPT NEG.		1-178 DPT
KE	EARLY C100	103 DPT NEG.		1-166 DPT
WA	EARLY CORE	76 DPT POS.	103-173 DPT	1-173 DPT
PA	EARLY C100	127 DPT POS.	1491-3644 DPT	1-3644 DPT
AN	EARLY 33C	179 DPT POS.	271-1489 DPT	1-1489 DPT

5

TABLE 9
SELECTED HCV E2 ANTIBODY POSITIVE SAMPLES

	<u>SAMPLE</u>	<u>C100 YEAST S/CO</u>	<u>C100 E. COLI S/CO</u>	<u>NS3 S/CO</u>	<u>CORE S/CO</u>	<u>E2 RIPA</u>
10	50	101.04	133.69	163.65	263.72	+
	121	1.28	4.77	172.65	291.82	+
	503	113.7	128.5	154.5	283.3	+
	505	130.6	143.8	133.4	186.1	-
	476	0.37	1.29	144.66	302.35	-
	728	-0.01	0.26	90.37	246.30	+

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: CASEY, JAMES M.
BODE, SUZANNE L.
ZECK, BILLY J.
YAMAGUCHI, JULIE
FRAIL, DONALD E.
DESAI, SURESH M.
DEVARE, SUSHIL G.

(ii) TITLE OF INVENTION: MAMMALIAN EXPRESSION SYSTEMS FOR HCV
PROTEINS

(iii) NUMBER OF SEQUENCES: 12

(iv) CORRESPONDENCE ADDRESS:

(A) ADDRESSEE: ABBOTT LABORATORIES D377/AP6D
(B) STREET: ONE ABBOTT PARK ROAD
(C) CITY: ABBOTT PARK
(D) STATE: IL
(E) COUNTRY: USA
(F) ZIP: 60064-3500

(v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:

(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:

(A) NAME: POREMBSKI, PRISCILLA E.
(B) REGISTRATION NUMBER: 33,207
(C) REFERENCE/DOCKET NUMBER: 5131.PC.01

(ix) TELECOMMUNICATION INFORMATION:

(A) TELEPHONE: 708-937-6365
(B) TELEFAX: 708-937-9556

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 3011 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

Met	Ser	Thr	Asn	Pro	Lys	Pro	Gln	Arg	Lys	Thr	Lys	Arg	Asn	Thr	Asn	1	5	10	15
Arg	Arg	Pro	Gln	Asp	Val	Lys	Phe	Pro	Gly	Gly	Gly	Gln	Ile	Val	Gly	20	25	30	
Gly	Val	Tyr	Leu	Leu	Pro	Arg	Arg	Gly	Pro	Arg	Leu	Gly	Val	Arg	Ala	35	40	45	
Thr	Arg	Lys	Thr	Ser	Glu	Arg	Ser	Gln	Pro	Arg	Gly	Arg	Arg	Gln	Pro	50	55	60	
Ile	Pro	Lys	Ala	Arg	Arg	Pro	Glu	Gly	Arg	Thr	Trp	Ala	Gln	Pro	Gly	65	70	75	80
Tyr	Pro	Trp	Pro	Leu	Tyr	Gly	Asn	Glu	Gly	Cys	Gly	Trp	Ala	Gly	Trp	85	90	95	
Leu	Leu	Ser	Pro	Arg	Gly	Ser	Arg	Pro	Ser	Trp	Gly	Pro	Thr	Asp	Pro	100	105	110	
Arg	Arg	Arg	Ser	Arg	Asn	Leu	Gly	Lys	Val	Ile	Asp	Thr	Leu	Thr	Cys	115	120	125	
Gly	Phe	Ala	Asp	Leu	Met	Gly	Tyr	Ile	Pro	Leu	Val	Gly	Ala	Pro	Leu	130	135	140	
Gly	Gly	Ala	Ala	Arg	Ala	Leu	Ala	His	Gly	Val	Arg	Val	Leu	Glu	Asp	145	150	155	160
Gly	Val	Asn	Tyr	Ala	Thr	Gly	Asn	Leu	Pro	Gly	Cys	Ser	Phe	Ser	Ile	165	170	175	
Phe	Leu	Leu	Ala	Leu	Leu	Ser	Cys	Leu	Thr	Val	Pro	Ala	Ser	Ala	Tyr	180	185	190	
Gln	Val	Arg	Asn	Ser	Ser	Gly	Leu	Tyr	His	Val	Thr	Asn	Asp	Cys	Pro	195	200	205	
Asn	Ser	Ser	Ile	Val	Tyr	Glu	Ala	Ala	Asp	Ala	Ile	Leu	His	Thr	Pro	210	215	220	
Gly	Cys	Val	Pro	Cys	Val	Arg	Glu	Gly	Asn	Ala	Ser	Arg	Cys	Trp	Val	225	230	235	240
Ala	Val	Thr	Pro	Thr	Val	Ala	Thr	Arg	Asp	Gly	Lys	Leu	Pro	Thr	Thr	245	250	255	
Gln	Leu	Arg	Arg	His	Ile	Asp	Leu	Leu	Val	Gly	Ser	Ala	Thr	Leu	Cys				

30

260	265	270
Ser Ala Leu Tyr Val Gly Asp	Leu Cys Gly Ser Val	Phe Leu Val Gly
275	280	285
Gln Leu Phe Thr Phe Ser Pro Arg Arg His Trp Thr Thr Gln Asp Cys		
290	295	300
Asn Cys Ser Ile Tyr Pro Gly His Ile Thr Gly His Arg Met Ala Trp		
305	310	315
Asp Met Met Met Asn Trp Ser Pro Thr Ala Ala Leu Val Val Ala Gln		
325	330	335
Leu Leu Arg Ile Pro Gln Ala Ile Leu Asp Met Ile Ala Gly Ala His		
340	345	350
Trp Gly Val Leu Ala Gly Ile Ala Tyr Phe Ser Met Val Gly Asn Trp		
355	360	365
Ala Lys Val Leu Val Val Leu Leu Leu Phe Ala Gly Val Asp Ala Glu		
370	375	380
Thr His Val Thr Gly Gly Ser Ala Gly His Thr Thr Ala Gly Leu Val		
385	390	395
Arg Leu Leu Ser Pro Gly Ala Lys Gln Asn Ile Gln Leu Ile Asn Thr		
405	410	415
Asn Gly Ser Trp His Ile Asn Ser Thr Ala Leu Asn Cys Asn Glu Ser		
420	425	430
Leu Asn Thr Gly Trp Leu Ala Gly Leu Phe Tyr His His Lys Phe Asn		
435	440	445
Ser Ser Gly Cys Pro Glu Arg Leu Ala Ser Cys Arg Arg Leu Thr Asp		
450	455	460
Phe Ala Gln Gly Gly Gly Pro Ile Ser Tyr Ala Asn Gly Ser Gly Leu		
465	470	475
Asp Glu Arg Pro Tyr Cys Trp His Tyr Pro Pro Arg Pro Cys Gly Ile		
485	490	495
Val Pro Ala Lys Ser Val Cys Gly Pro Val Tyr Cys Phe Thr Pro Ser		
500	505	510
Pro Val Val Val Gly Thr Thr Asp Arg Ser Gly Ala Pro Thr Tyr Ser		
515	520	525
Trp Gly Ala Asn Asp Thr Asp Val Phe Val Leu Asn Asn Thr Arg Pro		
530	535	540
Pro Leu Gly Asn Trp Phe Gly Cys Thr Trp Met Asn Ser Thr Gly Phe		
545	550	555
		560

Thr Lys Val Cys Gly Ala Pro Pro Cys Val Ile Gly Gly Val Gly Asn
 565 570 575
 Asn Thr Leu Leu Cys Pro Thr Asp Cys Phe Arg Lys His Pro Glu Ala
 580 585 590
 Thr Tyr Ser Arg Cys Gly Ser Gly Pro Trp Ile Thr Pro Arg Cys Met
 595 600 605
 Val Asp Tyr Pro Tyr Arg Leu Trp His Tyr Pro Cys Thr Ile Asn Tyr
 610 615 620
 Thr Ile Phe Lys Val Arg Met Tyr Val Gly Gly Val Glu His Arg Leu
 625 630 635 640
 Glu Ala Ala Cys Asn Trp Thr Arg Gly Glu Arg Cys Asp Leu Glu Asp
 645 650 655
 Arg Asp Arg Ser Glu Leu Ser Pro Leu Leu Leu Ser Thr Thr Gln Trp
 660 665 670
 Gln Val Leu Pro Cys Ser Phe Thr Thr Leu Pro Ala Leu Ser Thr Gly
 675 680 685
 Leu Ile His Leu His Gln Asn Ile Val Asp Val Gln Tyr Leu Tyr Gly
 690 695 700
 Val Gly Ser Ser Ile Ala Ser Trp Ala Ile Lys Trp Glu Tyr Val Val
 705 710 715 720
 Leu Leu Phe Leu Leu Leu Ala Asp Ala Arg Val Cys Ser Cys Leu Trp
 725 730 735
 Met Met Leu Leu Ile Ser Gln Ala Glu Ala Ala Leu Glu Asn Leu Val
 740 745 750
 Ile Leu Asn Ala Ala Ser Leu Ala Gly Thr His Gly Phe Val Ser Phe
 755 760 765
 Leu Val Phe Phe Cys Phe Ala Trp Tyr Leu Lys Gly Arg Trp Val Pro
 770 775 780
 Gly Ala Ala Tyr Ala Leu Tyr Gly Ile Trp Pro Leu Leu Leu Leu Leu
 785 790 795 800
 Leu Ala Leu Pro Gln Arg Ala Tyr Ala Leu Asp Thr Glu Val Ala Ala
 805 810 815
 Ser Cys Gly Gly Val Val Leu Val Gly Leu Met Ala Leu Thr Leu Ser
 820 825 830
 Pro Tyr Tyr Lys Arg Tyr Ile Ser Trp Cys Met Trp Trp Leu Gln Tyr
 835 840 845

Phe Leu Thr Arg Val Glu Ala Gln Leu His Val Trp Val Pro Pro Leu
 850 855 860
 Asn Val Arg Gly Gly Arg Asp Ala Val Ile Leu Leu Met Cys Ala Val
 865 870 875 880
 His Pro Thr Leu Val Phe Asp Ile Thr Lys Leu Leu Leu Ala Ile Phe
 885 890 895
 Gly Pro Leu Trp Ile Leu Gln Ala Ser Leu Leu Lys Val Pro Tyr Phe
 900 905 910
 Val Arg Val Gln Gly Leu Leu Arg Ile Cys Ala Leu Ala Arg Lys Ile
 915 920 925
 Ala Gly Gly His Tyr Val Gln Met Ile Phe Ile Lys Leu Gly Ala Leu
 930 935 940
 Thr Gly Thr Tyr Val Tyr Asn His Leu Thr Pro Leu Arg Asp Trp Ala
 945 950 955 960
 His Asn Gly Leu Arg Asp Leu Ala Val Ala Val Glu Pro Val Val Phe
 965 970 975
 Ser Arg Met Glu Thr Lys Leu Ile Thr Trp Gly Ala Asp Thr Ala Ala
 980 985 990
 Cys Gly Asp Ile Ile Asn Gly Leu Pro Val Ser Ala Arg Arg Gly Gln
 995 1000 1005
 Glu Ile Leu Leu Gly Pro Ala Asp Gly Met Val Ser Lys Gly Trp Arg
 1010 1015 1020
 Leu Leu Ala Pro Ile Thr Ala Tyr Ala Gln Gln Thr Arg Gly Leu Leu
 1025 1030 1035 1040
 Gly Cys Ile Ile Thr Ser Leu Thr Gly Arg Asp Lys Asn Gln Val Glu
 1045 1050 1055
 Gly Glu Val Gln Ile Val Ser Thr Ala Thr Gln Thr Phe Leu Ala Thr
 1060 1065 1070
 Cys Ile Asn Gly Val Cys Trp Thr Val Tyr His Gly Ala Gly Thr Arg
 1075 1080 1085
 Thr Ile Ala Ser Pro Lys Gly Pro Val Ile Gln Met Tyr Thr Asn Val
 1090 1095 1100
 Asp Gln Asp Leu Val Gly Trp Pro Ala Pro Gln Gly Ser Arg Ser Leu
 1105 1110 1115 1120
 Thr Pro Cys Thr Cys Gly Ser Ser Asp Leu Tyr Leu Val Thr Arg His
 1125 1130 1135
 Ala Asp Val Ile Pro Val Arg Arg Gln Gly Asp Ser Arg Gly Ser Leu

33

1140	1145	1150
Leu Ser Pro Arg Pro Ile Ser Tyr Leu Lys Gly Ser Ser Gly Gly Pro		
1155	1160	1165
Leu Leu Cys Pro Ala Gly His Ala Val Gly Leu Phe Arg Ala Ala Val		
1170	1175	1180
Cys Thr Arg Gly Val Ala Lys Ala Val Asp Phe Ile Pro Val Glu Asn		
1185	1190	1195 1200
Leu Glu Thr Thr Met Arg Ser Pro Val Phe Thr Asp Asn Ser Ser Pro		
1205	1210	1215
Pro Ala Val Pro Gln Ser Phe Gln Val Ala His Leu His Ala Pro Thr		
1220	1225	1230
Gly Ser Gly Lys Ser Thr Lys Val Pro Ala Ala Tyr Ala Ala Gln Gly		
1235	1240	1245
Tyr Lys Val Leu Val Leu Asn Pro Ser Val Ala Ala Thr Leu Gly Phe		
1250	1255	1260
Gly Ala Tyr Met Ser Lys Ala His Gly Val Asp Pro Asn Ile Arg Thr		
1265	1270	1275 1280
Gly Val Arg Thr Ile Thr Thr Gly Ser Pro Ile Thr Tyr Ser Thr Tyr		
1285	1290	1295
Gly Lys Phe Leu Ala Asp Gly Gly Cys Ser Gly Gly Ala Tyr Asp Ile		
1300	1305	1310
Ile Ile Cys Asp Glu Cys His Ser Thr Asp Ala Thr Ser Ile Leu Gly		
1315	1320	1325
Ile Gly Thr Val Leu Asp Gln Ala Glu Thr Ala Gly Ala Arg Leu Val		
1330	1335	1340
Val Leu Ala Thr Ala Thr Pro Pro Gly Ser Val Thr Val Pro His Pro		
1345	1350	1355 1360
Asn Ile Glu Glu Val Ala Leu Ser Thr Thr Gly Glu Ile Pro Phe Tyr		
1365	1370	1375
Gly Lys Ala Ile Pro Leu Glu Val Ile Lys Gly Gly Arg His Leu Ile		
1380	1385	1390
Phe Cys His Ser Lys Lys Lys Cys Asp Glu Leu Ala Ala Lys Leu Val		
1395	1400	1405
Ala Leu Gly Ile Asn Ala Val Ala Tyr Tyr Arg Gly Leu Asp Val Ser		
1410	1415	1420
Val Ile Pro Ala Ser Gly Asp Val Val Val Val Ser Thr Asp Ala Leu		
1425	1430	1435 1440

Met Thr Gly Phe Thr Gly Asp Phe Asp Pro Val Ile Asp Cys Asn Thr
 1445 1450 1455
 Cys Val Thr Gln Thr Val Asp Phe Ser Leu Asp Pro Thr Phe Thr Ile
 1460 1465 1470
 Glu Thr Thr Thr Leu Pro Gln Asp Ala Val Ser Arg Thr Gln Arg Arg
 1475 1480 1485
 Gly Arg Thr Gly Arg Gly Lys Pro Gly Ile Tyr Arg Phe Val Ala Pro
 1490 1495 1500
 Gly Glu Arg Pro Ser Gly Met Phe Asp Ser Ser Val Leu Cys Glu Cys
 1505 1510 1515 1520
 Tyr Asp Ala Gly Cys Ala Trp Tyr Glu Leu Thr Pro Ala Glu Thr Thr
 1525 1530 1535
 Val Arg Leu Arg Ala Tyr Met Asn Thr Pro Gly Leu Pro Val Cys Gln
 1540 1545 1550
 Asp His Leu Glu Phe Trp Glu Gly Val Phe Thr Gly Leu Thr His Ile
 1555 1560 1565
 Asp Ala His Phe Leu Ser Gln Thr Lys Gln Ser Gly Glu Asn Phe Pro
 1570 1575 1580
 Tyr Leu Val Ala Tyr Gln Ala Thr Val Cys Ala Arg Ala Gln Ala Pro
 1585 1590 1595 1600
 Pro Pro Ser Trp Asp Gln Met Trp Lys Cys Leu Ile Arg Leu Lys Pro
 1605 1610 1615
 Thr Leu His Gly Pro Thr Pro Leu Leu Tyr Arg Leu Gly Ala Val Gln
 1620 1625 1630
 Asn Glu Ile Thr Leu Thr His Pro Val Thr Lys Tyr Ile Met Thr Cys
 1635 1640 1645
 Met Ser Ala Asn Pro Glu Val Val Thr Ser Thr Trp Val Leu Val Gly
 1650 1655 1660
 Gly Val Leu Ala Ala Leu Ala Ala Tyr Cys Leu Ser Thr Gly Cys Val
 1665 1670 1675 1680
 Val Ile Val Gly Arg Ile Val Leu Ser Gly Lys Pro Ala Ile Ile Pro
 1685 1690 1695
 Asp Arg Glu Val Leu Tyr Gln Glu Phe Asp Glu Met Glu Glu Cys Ser
 1700 1705 1710
 Gln His Leu Pro Tyr Ile Glu Gln Gly Met Met Leu Ala Glu Gln Phe
 1715 1720 1725

Lys Gln Glu Ala Leu Gly Leu Leu Gln Thr Ala Ser Arg Gln Ala Glu
 1730 1735 1740
 Val Ile Thr Pro Ala Val Gln Thr Asn Trp Gln Lys Leu Glu Ala Phe
 1745 1750 1755 1760
 Trp Ala Lys His Met Trp Asn Phe Ile Ser Gly Thr Gln Tyr Leu Ala
 1765 1770 1775
 Gly Leu Ser Thr Leu Pro Gly Asn Pro Ala Ile Ala Ser Leu Met Ala
 1780 1785 1790
 Phe Thr Ala Ala Val Thr Ser Pro Leu Thr Thr Ser Gln Thr Leu Leu
 1795 1800 1805
 Phe Asn Ile Leu Gly Gly Trp Val Ala Ala Gln Leu Ala Ala Pro Gly
 1810 1815 1820
 Ala Ala Thr Ala Phe Val Gly Ala Gly Leu Ala Gly Ala Ala Ile Gly
 1825 1830 1835 1840
 Ser Val Gly Leu Gly Lys Val Leu Val Asp Ile Leu Ala Gly Tyr Gly
 1845 1850 1855
 Ala Gly Val Ala Gly Ala Leu Val Ala Phe Lys Ile Met Ser Gly Glu
 1860 1865 1870
 Val Pro Ser Thr Glu Asp Leu Val Asn Leu Leu Pro Ala Ile Leu Ser
 1875 1880 1885
 Pro Gly Ala Leu Val Val Gly Val Val Cys Ala Ala Ile Leu Arg Arg
 1890 1895 1900
 His Val Gly Pro Gly Glu Gly Ala Val Gln Trp Met Asn Arg Leu Ile
 1905 1910 1915 1920
 Ala Phe Ala Ser Arg Gly Asn His Val Ser Pro Thr His Tyr Val Pro
 1925 1930 1935
 Glu Ser Asp Ala Ala Ala Arg Val Thr Ala Ile Leu Ser Asn Leu Thr
 1940 1945 1950
 Val Thr Gln Leu Leu Arg Arg Leu His Gln Trp Ile Gly Ser Glu Cys
 1955 1960 1965
 Thr Thr Pro Cys Ser Gly Ser Trp Leu Arg Asp Ile Trp Asp Trp Ile
 1970 1975 1980
 Cys Glu Val Leu Ser Asp Phe Lys Thr Trp Leu Lys Ala Lys Leu Met
 1985 1990 1995 2000
 Pro Gln L u Pro Gly Ile Pro Phe Val Ser Cys Gln Arg Gly Tyr Arg
 2005 2010 2015
 Gly Val Trp Arg Gly Asp Gly Ile Met His Thr Arg Cys His Cys Gly

36

2020	2025	2030
Ala Glu Ile Thr Gly His Val Lys Asn Gly Thr Met Arg Ile Val Gly 2035	2040	2045
Pro Arg Thr Cys Arg Asn Met Trp Ser Gly Thr Phe Pro Ile Asn Ala 2050	2055	2060
Tyr Thr Thr Gly Pro Cys Thr Pro Leu Pro Ala Pro Asn Tyr Lys Phe 2065	2070	2075 2080
Ala Leu Trp Arg Val Ser Ala Glu Glu Tyr Val Glu Ile Arg Arg Val 2085	2090	2095
Gly Asp Phe His Tyr Val Ser Gly Met Thr Thr Asp Asn Leu Lys Cys 2100	2105	2110
Pro Cys Gln Ile Pro Ser Pro Glu Phe Phe Thr Glu Leu Asp Gly Val 2115	2120	2125
Arg Leu His Arg Phe Ala Pro Pro Cys Lys Pro Leu Leu Arg Glu Glu 2130	2135	2140
Val Ser Phe Arg Val Gly Leu His Glu Tyr Pro Val Gly Ser Gln Leu 2145	2150	2155 2160
Pro Cys Glu Pro Glu Pro Asp Val Ala Val Leu Thr Ser Met Leu Thr 2165	2170	2175
Asp Pro Ser His Ile Thr Ala Glu Ala Ala Gly Arg Arg Leu Ala Arg 2180	2185	2190
Gly Ser Pro Pro Ser Met Ala Ser Ser Ser Ala Ser Gln Leu Ser Ala 2195	2200	2205
Pro Ser Leu Lys Ala Thr Cys Thr Thr Asn His Asp Ser Pro Asp Ala 2210	2215	2220
Glu Leu Ile Glu Ala Asn Leu Leu Trp Arg Gln Glu Met Gly Gly Asn 2225	2230	2235 2240
Ile Thr Arg Val Glu Ser Glu Asn Lys Val Val Ile Leu Asp Ser Phe 2245	2250	2255
Asp Pro Leu Val Ala Glu Glu Asp Glu Arg Glu Val Ser Val Pro Ala 2260	2265	2270
Glu Ile Leu Arg Lys Ser Gln Arg Phe Ala Arg Ala Leu Pro Val Trp 2275	2280	2285
Ala Arg Pro Asp Tyr Asn Pro Pro Leu Ile Glu Thr Trp Lys Glu Pro 2290	2295	2300
Asp Tyr Glu Pro Pro Val Val His Gly Cys Pro Leu Pro Pro Pro Arg 2305	2310	2315 2320

Ser Pro Pro Val Pro Pro Pro Arg Lys Lys Arg Thr Val Val Leu Thr
 2325 2330 2335
 Glu Ser Thr Leu Ser Thr Ala Leu Ala Glu Leu Ala Thr Lys Ser Phe
 2340 2345 2350
 Gly Ser Ser Ser Thr Ser Gly Ile Thr Gly Asp Asn Thr Thr Thr Ser
 2355 2360 2365
 Ser Glu Pro Ala Pro Ser Gly Cys Pro Pro Asp Ser Asp Val Glu Ser
 2370 2375 2380
 Tyr Ser Ser Met Pro Pro Leu Glu Gly Glu Pro Gly Asp Pro Asp Phe
 2385 2390 2395 2400
 Ser Asp Gly Ser Trp Ser Thr Val Ser Ser Gly Ala Asp Thr Glu Asp
 2405 2410 2415
 Val Val Cys Cys Ser Met Ser Tyr Ser Trp Thr Gly Ala Leu Val Thr
 2420 2425 2430
 Pro Cys Ala Ala Glu Glu Gln Lys Leu Pro Ile Asn Ala Leu Ser Asn
 2435 2440 2445
 Ser Leu Leu Arg His His Asn Leu Val Tyr Ser Thr Thr Ser Arg Ser
 2450 2455 2460
 Ala Cys Gln Arg Gln Lys Lys Val Thr Phe Asp Arg Leu Gln Val Leu
 2465 2470 2475 2480
 Asp Ser His Tyr Gln Asp Val Leu Lys Glu Val Lys Ala Ala Ala Ser
 2485 2490 2495
 Arg Val Lys Ala Asn Leu Leu Ser Val Glu Glu Ala Cys Ser Leu Thr
 2500 2505 2510
 Pro Pro His Ser Ala Lys Ser Lys Phe Gly Tyr Gly Ala Lys Asp Val
 2515 2520 2525
 Arg Cys His Ala Arg Lys Ala Val Ala His Ile Asn Ser Val Trp Lys
 2530 2535 2540
 Asp Leu Leu Glu Asp Ser Val Thr Pro Ile Asp Thr Thr Ile Met Ala
 2545 2550 2555 2560
 Lys Asn Glu Val Phe Cys Val Gln Pro Glu Lys Gly Gly Arg Lys Pro
 2565 2570 2575
 Ala Arg Leu Ile Val Phe Pro Asp Leu Gly Val Arg Val Cys Glu Lys
 2580 2585 2590
 Met Ala Leu Tyr Asp Val Val Ser Lys Leu Pro Leu Ala Val Met Gly
 2595 2600 2605

Ser Ser Tyr Gly Phe Gln Tyr Ser Pro Gly Gln Arg Val Glu Phe Leu
 2610 2615 2620
 Val Gln Ala Trp Lys Ser Lys Lys Thr Pro Met Gly Phe Ser Tyr Asp
 2625 2630 2635 2640
 Thr Arg Cys Phe Asp Ser Thr Val Thr Glu Ser Asp Ile Arg Thr Glu
 2645 2650 2655
 Glu Ala Ile Tyr Gln Cys Cys Asp Leu Asp Pro Gln Ala Arg Val Ala
 2660 2665 2670
 Ile Lys Ser Leu Thr Glu Arg Leu Tyr Val Gly Gly Pro Leu Thr Asn
 2675 2680 2685
 Ser Arg Gly Glu Asn Cys Gly Tyr Arg Arg Cys Arg Ala Ser Gly Val
 2690 2695 2700
 Leu Thr Thr Ser Cys Gly Asn Thr Leu Thr Cys Tyr Ile Lys Ala Arg
 2705 2710 2715 2720
 Ala Ala Cys Arg Ala Ala Gly Leu Gln Asp Arg Thr Met Leu Val Cys
 2725 2730 2735
 Gly Asp Asp Leu Val Val Ile Cys Glu Ser Ala Gly Val Gln Glu Asp
 2740 2745 2750
 Ala Ala Ser Leu Arg Ala Phe Thr Glu Ala Met Thr Arg Tyr Ser Ala
 2755 2760 2765
 Pro Pro Gly Asp Pro Pro Gln Pro Glu Tyr Asp Leu Glu Leu Ile Thr
 2770 2775 2780
 Ser Cys Ser Ser Asn Val Ser Val Ala His Asp Gly Ala Gly Lys Arg
 2785 2790 2795 2800
 Val Tyr Tyr Leu Thr Arg Asp Pro Thr Thr Pro Leu Ala Arg Ala Ala
 2805 2810 2815
 Trp Glu Thr Ala Arg His Thr Pro Val Asn Ser Trp Leu Gly Asn Ile
 2820 2825 2830
 Ile Met Phe Ala Pro Thr Leu Trp Ala Arg Met Ile Leu Met Thr His
 2835 2840 2845
 Phe Phe Ser Val Leu Ile Ala Arg Asp Gln Phe Glu Gln Ala Leu Asn
 2850 2855 2860
 Cys Glu Ile Tyr Gly Ala Cys Tyr Ser Ile Glu Pro Leu Asp Leu Pro
 2865 2870 2875 2880
 Pro Ile Ile Gln Arg Leu His Gly Leu Ser Ala Phe Ser Leu His Ser
 2885 2890 2895
 Tyr Ser Pro Gly Glu Ile Asn Arg Val Ala Ala Cys Leu Arg Lys Leu

39

2900	2905	2910
Gly Val Pro Pro Leu Arg Ala Trp Lys His Arg Ala Arg Ser Val Arg 2915 2920 2925		
Ala Arg Leu Leu Ser Arg Gly Gly Arg Ala Ala Ile Cys Gly Lys Tyr 2930 2935 2940		
Leu Phe Asn Trp Ala Val Arg Thr Lys Pro Lys Leu Thr Pro Ile Ala 2945 2950 2955 2960		
Ala Ala Gly Arg Leu Asp Leu Ser Gly Trp Phe Thr Ala Gly Tyr Ser 2965 2970 2975		
Gly Gly Asp Ile Tyr His Ser Val Ser His Ala Arg Pro Arg Trp Ser 2980 2985 2990		
Trp Phe Cys Leu Leu Leu Leu Ala Ala Gly Val Gly Ile Tyr Leu Leu 2995 3000 3005		
Pro Asn Arg 3010		

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 3011 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION SEQ ID NO:2:

Met	Ser	Thr	Asn	Pro	Lys	Pro	Gln	Arg	Lys	Thr	Lys	Arg	Asn	Thr	Asn
1				5					10					15	
Arg	Arg	Pro	Gln	Asp	Val	Lys	Phe	Pro	Gly	Gly	Gly	Gln	Ile	Val	Gly
			20					25					30		
Gly	Val	Tyr	Leu	Leu	Pro	Arg	Arg	Gly	Pro	Arg	Leu	Gly	Val	Arg	Ala
		35					40					45			
Thr	Arg	Lys	Thr	Ser	Glu	Arg	Ser	Gln	Pro	Arg	Gly	Arg	Arg	Gln	Pro
	50					55					60				
Ile	Pro	Lys	Ala	Arg	Arg	Pro	Glu	Gly	Arg	Thr	Trp	Ala	Gln	Pro	Gly
65					70					75					80
Tyr	Pro	Trp	Pro	Leu	Tyr	Gly	Asn	Glu	Gly	Cys	Gly	Trp	Ala	Gly	Trp
				85					90					95	

40

Leu Leu Ser Pro Arg Gly Ser Arg Pro Ser Trp Gly Pro Thr Asp Pro
 100 105 110
 Arg Arg Arg Ser Arg Asn Leu Gly Lys Val Ile Asp Thr Leu Thr Cys
 115 120 125
 Gly Phe Ala Asp Leu Met Gly Tyr Ile Pro Leu Val Gly Ala Pro Leu
 130 135 140
 Gly Gly Ala Ala Arg Ala Leu Ala His Gly Val Arg Val Leu Glu Asp
 145 150 155 160
 Gly Val Asn Tyr Ala Thr Gly Asn Leu Pro Gly Cys Ser Phe Ser Ile
 165 170 175
 Phe Leu Leu Ala Leu Leu Ser Cys Leu Thr Val Pro Ala Ser Ala Tyr
 180 185 190
 Gln Val Arg Asn Ser Ser Gly Leu Tyr His Val Thr Asn Asp Cys Pro
 195 200 205
 Asn Ser Ser Ile Val Tyr Glu Thr Ala Asp Thr Ile Leu His Ser Pro
 210 215 220
 Gly Cys Val Pro Cys Val Arg Glu Gly Asn Thr Ser Lys Cys Trp Val
 225 230 235 240
 Ala Val Ala Pro Thr Val Thr Thr Arg Asp Gly Lys Leu Pro Ser Thr
 245 250 255
 Gln Leu Arg Arg His Ile Asp Leu Leu Val Gly Ser Ala Thr Leu Cys
 260 265 270
 Ser Ala Leu Tyr Val Gly Asp Leu Cys Gly Ser Val Phe Leu Val Ser
 275 280 285
 Gln Leu Phe Thr Phe Ser Pro Arg Arg His Trp Thr Thr Gln Asp Cys
 290 295 300
 Asn Cys Ser Ile Tyr Pro Gly His Ile Thr Gly His Arg Met Ala Trp
 305 310 315 320
 Asp Met Met Met Asn Trp Ser Pro Thr Thr Ala Leu Val Val Ala Gln
 325 330 335
 Leu Leu Arg Ile Pro Gln Ala Ile Leu Asp Met Ile Ala Gly Ala His
 340 345 350
 Trp Gly Val Leu Ala Gly Ile Ala Tyr Phe Ser Met Val Gly Asn Trp
 355 360 365
 Ala Lys Val Leu Val Val Leu Leu Leu Phe Ser Gly Val Asp Ala Ala
 370 375 380
 Thr Tyr Thr Thr Gly Gly Ser Val Ala Arg Thr Thr His Gly Leu Ser

41

385	390	395	400
Ser Leu Phe Ser Gln Gly Ala Lys Gln Asn Ile Gln Leu Ile Asn Thr	405	410	415
Asn Gly Ser Trp His Ile Asn Arg Thr Ala Leu Asn Cys Asn Ala Ser	420	425	430
Leu Asp Thr Gly Trp Val Ala Gly Leu Phe Tyr Tyr His Lys Phe Asn	435	440	445
Ser Ser Gly Cys Pro Glu Arg Met Ala Ser Cys Arg Pro Leu Ala Asp	450	455	460
Phe Asp Gln Gly Trp Gly Pro Ile Ser Tyr Thr Asn Gly Ser Gly Pro	465	470	475
Glu His Arg Pro Tyr Cys Trp His Tyr Pro Pro Lys Pro Cys Gly Ile	485	490	495
Val Pro Ala Gln Ser Val Cys Gly Pro Val Tyr Cys Phe Thr Pro Ser	500	505	510
Pro Val Val Val Gly Thr Thr Asp Lys Ser Gly Ala Pro Thr Tyr Thr	515	520	525
Trp Gly Ser Asn Asp Thr Asp Val Phe Val Leu Asn Asn Thr Arg Pro	530	535	540
Pro Pro Gly Asn Trp Phe Gly Cys Thr Trp Met Asn Ser Ser Gly Phe	545	550	555
Thr Lys Val Cys Gly Ala Pro Pro Cys Val Ile Gly Gly Ala Gly Asn	565	570	575
Asn Thr Leu His Cys Pro Thr Asp Cys Phe Arg Lys His Pro Glu Ala	580	585	590
Thr Tyr Ser Arg Cys Gly Ser Gly Pro Trp Ile Thr Pro Arg Cys Leu	595	600	605
Val His Tyr Pro Tyr Arg Leu Trp His Tyr Pro Cys Thr Ile Asn Tyr	610	615	620
Thr Leu Phe Lys Val Arg Met Tyr Val Gly Gly Val Glu His Arg Leu	625	630	635
Glu Val Ala Cys Asn Trp Thr Arg Gly Glu Arg Cys Asp Leu Asp Asp	645	650	655
Arg Asp Arg Ser Glu Leu Ser Pro Leu Leu Ser Thr Thr Gln Trp	660	665	670
Gln Val Leu Pro Cys Ser Phe Thr Thr Leu Pro Ala Leu Thr Thr Gly	675	680	685

Leu Ile His Leu His Gln Asn Ile Val Asp Val Gln Tyr Leu Tyr Gly
 690 695 700
 Val Gly Ser Ser Ile Val Ser Trp Ala Ile Lys Trp Glu Tyr Val Ile
 705 710 715 720
 Leu Leu Phe Leu Leu Leu Ala Asp Ala Arg Ile Cys Ser Cys Leu Trp
 725 730 735
 Met Met Leu Leu Ile Ser Gln Ala Glu Ala Ala Leu Glu Asn Leu Val
 740 745 750
 Leu Leu Asn Ala Ala Ser Leu Ala Gly Thr His Gly Leu Val Ser Phe
 755 760 765
 Leu Val Phe Phe Cys Phe Ala Trp Tyr Leu Lys Gly Lys Trp Val Pro
 770 775 780
 Gly Val Ala Tyr Ala Phe Tyr Gly Met Trp Pro Phe Leu Leu Leu Leu
 785 790 795 800
 Leu Ala Leu Pro Gln Arg Ala Tyr Ala Leu Asp Thr Glu Met Ala Ala
 805 810 815
 Ser Cys Gly Gly Val Val Leu Val Gly Leu Met Ala Leu Thr Leu Ser
 820 825 830
 Pro His Tyr Lys Arg Tyr Ile Cys Trp Cys Val Trp Trp Leu Gln Tyr
 835 840 845
 Phe Leu Thr Arg Ala Glu Ala Leu Leu His Gly Trp Val Pro Pro Leu
 850 855 860
 Asn Val Arg Gly Gly Arg Asp Ala Val Ile Leu Leu Met Cys Val Val
 865 870 875 880
 His Pro Ala Leu Val Phe Asp Ile Thr Lys Leu Leu Leu Ala Val Leu
 885 890 895
 Gly Pro Leu Trp Ile Leu Gln Thr Ser Leu Leu Lys Val Pro Tyr Phe
 900 905 910
 Val Arg Val Gln Gly Leu Leu Arg Ile Cys Ala Leu Ala Arg Lys Met
 915 920 925
 Ala Gly Gly His Tyr Val Gln Met Val Thr Ile Lys Met Gly Ala Leu
 930 935 940
 Ala Gly Thr Tyr Val Tyr Asn His Leu Thr Pro Leu Arg Asp Trp Ala
 945 950 955 960
 His Asn Gly Leu Arg Asp Leu Ala Val Ala Val Glu Pro Val Val Phe
 965 970 975

Ser Gln Met Glu Thr Lys Leu Ile Thr Trp Gly Ala Asp Thr Ala Ala
 980 985 990
 Cys Gly Asp Ile Ile Asn Gly Leu Pro Val Ser Ala Arg Arg Gly Arg
 995 1000 1005
 Glu Ile Leu Leu Gly Pro Ala Asp Gly Met Val Ser Lys Gly Trp Arg
 1010 1015 1020
 Leu Leu Ala Pro Ile Thr Ala Tyr Ala Gln Gln Thr Arg Gly Leu Leu
 1025 1030 1035 1040
 Gly Cys Ile Ile Thr Ser Leu Thr Gly Arg Asp Lys Asn Gln Val Glu
 1045 1050 1055
 Gly Glu Val Gln Ile Val Ser Thr Ala Ala Gln Thr Phe Leu Ala Thr
 1060 1065 1070
 Cys Ile Asn Gly Val Cys Trp Thr Val Tyr His Gly Ala Gly Thr Arg
 1075 1080 1085
 Thr Ile Ala Ser Pro Lys Gly Pro Val Ile Gln Met Tyr Thr Asn Val
 1090 1095 1100
 Asp Arg Asp Leu Val Gly Trp Pro Ala Pro Gln Gly Ala Arg Ser Leu
 1105 1110 1115 1120
 Thr Pro Cys Thr Cys Gly Ser Ser Asp Leu Tyr Leu Val Thr Arg His
 1125 1130 1135
 Ala Asp Val Ile Pro Val Arg Arg Arg Gly Asp Ser Arg Gly Ser Leu
 1140 1145 1150
 Leu Ser Pro Arg Pro Ile Ser Tyr Leu Lys Gly Ser Ser Gly Gly Pro
 1155 1160 1165
 Leu Leu Cys Pro Ala Gly His Ala Val Gly Ile Phe Arg Ala Ala Val
 1170 1175 1180
 Cys Thr Arg Gly Val Ala Lys Ala Val Asp Phe Ile Pro Val Glu Ser
 1185 1190 1195 1200
 Leu Glu Thr Thr Met Arg Ser Pro Val Phe Thr Asp Asn Ser Ser Pro
 1205 1210 1215
 Pro Ala Val Pro Gln Ser Phe Gln Val Ala His Leu His Ala Pro Thr
 1220 1225 1230
 Gly Ser Gly Lys Ser Thr Lys Val Pro Ala Ala Tyr Ala Ala Gln Gly
 1235 1240 1245
 Tyr Lys Val Leu Val Leu Asn Pro Ser Val Ala Ala Thr Leu Gly Phe
 1250 1255 1260
 Gly Ala Tyr Met Ser Lys Ala His Gly Ile Asp Pro Asn Ile Arg Thr

1265	1270	1275	1280
Gly Val Arg Thr Ile Thr Thr Gly Ser Pro Ile Thr Tyr Ser Thr Tyr	1285	1290	1295
Gly Lys Phe Leu Ala Asp Gly Gly Cys Ser Gly Gly Ala Tyr Asp Ile	1300	1305	1310
Ile Ile Cys Asp Glu Cys His Ser Thr Asp Ala Thr Ser Ile Leu Gly	1315	1320	1325
Ile Gly Thr Val Leu Asp Gln Ala Glu Thr Ala Gly Ala Arg Leu Val	1330	1335	1340
Val Leu Ala Thr Ala Thr Pro Pro Gly Ser Val Thr Val Pro His Pro	1345	1350	1355
Asn Ile Glu Glu Val Ala Leu Ser Thr Thr Gly Glu Ile Pro Phe Tyr	1365	1370	1375
Gly Lys Ala Ile Pro Leu Glu Ala Ile Lys Gly Gly Arg His Leu Ile	1380	1385	1390
Phe Cys His Ser Lys Lys Lys Cys Asp Glu Leu Ala Ala Lys Leu Val	1395	1400	1405
Thr Leu Gly Ile Asn Ala Val Ala Tyr Tyr Arg Gly Leu Asp Val Ser	1410	1415	1420
Val Ile Pro Thr Ser Gly Asp Val Val Val Val Ala Thr Asp Ala Leu	1425	1430	1435
Met Thr Gly Phe Thr Gly Asp Phe Asp Ser Val Ile Asp Cys Asn Thr	1445	1450	1455
Cys Val Thr Gln Ala Val Asp Phe Ser Leu Asp Pro Thr Phe Thr Ile	1460	1465	1470
Glu Thr Thr Thr Leu Pro Gln Asp Ala Val Ser Arg Thr Gln Arg Arg	1475	1480	1485
Gly Arg Thr Gly Arg Gly Lys Pro Gly Ile Tyr Arg Phe Val Ala Pro	1490	1495	1500
Gly Glu Arg Pro Ser Gly Met Phe Asp Ser Ser Val Leu Cys Glu Cys	1505	1510	1515
Tyr Asp Ala Gly Cys Ala Trp Tyr Glu Leu Thr Pro Ala Glu Thr Thr	1525	1530	1535
Val Arg Leu Arg Ala Tyr Met Asn Thr Pro Gly Leu Pro Val Cys Gln	1540	1545	1550
Asp His Leu Glu Phe Trp Glu Gly Val Phe Thr Gly Leu Thr His Ile	1555	1560	1565

Asp Ala His Phe Leu Ser Gln Thr Lys Gln Ser Gly Glu Asn Leu Pro
 1570 1575 1580
 Tyr Leu Val Ala Tyr Gln Ala Thr Val Cys Ala Arg Ala Gln Ala Pro
 1585 1590 1595 1600
 Pro Pro Ser Trp Asp Gln Met Trp Lys Cys Leu Ile Arg Leu Lys Pro
 1605 1610 1615
 Thr Leu His Gly Pro Thr Pro Leu Leu Tyr Arg Leu Gly Ala Val Gln
 1620 1625 1630
 Asn Glu Val Thr Leu Thr His Pro Ile Thr Lys Tyr Ile Met Thr Cys
 1635 1640 1645
 Met Ser Ala Asp Leu Glu Val Val Thr Ser Thr Trp Val Leu Val Gly
 1650 1655 1660
 Gly Val Leu Ala Ala Leu Ala Ala Tyr Cys Leu Ser Thr Gly Cys Val
 1665 1670 1675 1680
 Val Ile Val Gly Arg Ile Val Leu Ser Gly Lys Pro Ala Ile Ile Pro
 1685 1690 1695
 Asp Arg Glu Val Leu Tyr Arg Glu Phe Asp Glu Met Glu Glu Cys Ser
 1700 1705 1710
 Gln His Leu Pro Tyr Ile Glu Gln Gly Met Met Leu Ala Glu Gln Phe
 1715 1720 1725
 Lys Gln Lys Ala Leu Gly Leu Leu Gln Thr Ala Ser His Gln Ala Glu
 1730 1735 1740
 Val Ile Ala Pro Ala Val Gln Thr Asn Trp Gln Arg Leu Glu Thr Phe
 1745 1750 1755 1760
 Trp Ala Lys His Met Trp Asn Phe Ile Ser Gly Ile Gln Tyr Leu Ala
 1765 1770 1775
 Gly Leu Ser Thr Leu Pro Gly Asn Pro Ala Ile Ala Ser Leu Met Ala
 1780 1785 1790
 Phe Thr Ala Ala Val Thr Ser Pro Leu Thr Thr Ser Gln Thr Leu Leu
 1795 1800 1805
 Phe Asn Ile Leu Gly Gly Trp Val Ala Ala Gln Leu Ala Ala Pro Ser
 1810 1815 1820
 Ala Ala Thr Ala Phe Val Gly Ala Gly Leu Ala Gly Ala Ala Ile Gly
 1825 1830 1835 1840
 Ser Val Gly Leu Gly Lys Val Leu Val Asp Ile Leu Ala Gly Tyr Gly
 1845 1850 1855

46

Ala Gly Val Ala Gly Ala Leu Val Ala Phe Lys Ile Met Ser Gly Glu
1860 1865 1870

Val Pro Ser Thr Glu Asp Leu Val Asn Leu Leu Pro Ala Ile Leu Ser
1875 1880 1885

Pro Gly Ala Leu Val Val Gly Val Val Cys Ala Ala Ile Leu Arg Arg
1890 1895 1900

His Val Gly Pro Gly Glu Gly Ala Val Gln Trp Met Asn Arg Leu Ile
1905 1910 1915 1920

Ala Phe Ala Ser Arg Gly Asn His Val Ser Pro Thr His Tyr Val Pro
1925 1930 1935

Gly Ser Asp Ala Ala Ala Arg Val Thr Ala Ile Leu Ser Ser Leu Thr
1940 1945 1950

Val Thr Gln Leu Leu Arg Arg Leu His Gln Trp Val Ser Ser Glu Cys
1955 1960 1965

Thr Thr Pro Cys Ser Gly Ser Trp Leu Arg Asp Ile Trp Asp Trp Ile
1970 1975 1980

Cys Glu Val Leu Ser Asp Phe Lys Thr Trp Leu Lys Ala Lys Leu Met
1985 1990 1995 2000

Pro Gln Leu Pro Gly Ile Pro Phe Val Ser Cys Gln Arg Gly Tyr Lys
2005 2010 2015

Gly Val Trp Arg Gly Asp Gly Ile Met His Thr Arg Cys His Cys Gly
2020 2025 2030

Ala Glu Ile Ala Gly His Val Lys Asn Gly Thr Met Arg Ile Val Gly
2035 2040 2045

Pro Lys Thr Cys Arg Asn Met Trp Ser Gly Thr Phe Pro Ile Asn Ala
2050 2055 2060

Tyr Thr Thr Gly Pro Cys Thr Pro Leu Pro Ala Pro Asn Tyr Lys Phe
2065 2070 2075 2080

Ala Leu Trp Arg Val Ser Ala Glu Glu Tyr Val Glu Ile Arg Gln Val
2085 2090 2095

Gly Asp Phe His Tyr Val Thr Gly Met Thr Ala Asp Asn Leu Lys Cys
2100 2105 2110

Pro Cys Gln Val Pro Ser Pro Glu Phe Phe Thr Glu Leu Asp Gly Val
2115 2120 2125

Arg Leu His Arg Phe Ala Pro Pro Cys Lys Pro Leu Leu Arg Asp Glu
2130 2135 2140

Val Ser Phe Arg Val Gly Leu His Asp Tyr Pro Val Gly Ser Gln Leu

2145	2150	2155	2160
Pro Cys Glu Pro Glu Pro Asp Val Ala Val Leu Thr Ser Met Leu Thr	2165	2170	2175
Asp Pro Ser His Ile Thr Ala Glu Thr Ala Gly Arg Arg Leu Ala Arg	2180	2185	2190
Gly Ser Pro Pro Ser Met Ala Ser Ser Ser Ala Ser Gln Leu Ser Ala	2195	2200	2205
Pro Ser Leu Lys Ala Thr Cys Thr Thr Asn His Asp Ser Pro Asp Ala	2210	2215	2220
Glu Leu Leu Glu Ala Asn Leu Leu Trp Arg Gln Glu Met Gly Gly Asn	2225	2230	2235
Ile Thr Arg Val Glu Ser Glu Asn Lys Val Val Val Leu Asp Ser Phe	2245	2250	2255
Asp Pro Leu Val Ala Glu Glu Asp Glu Arg Glu Val Ser Val Pro Ala	2260	2265	2270
Glu Ile Leu Arg Lys Ser Arg Arg Phe Ala Gln Ala Leu Pro Ser Trp	2275	2280	2285
Ala Arg Pro Asp Tyr Asn Pro Pro Leu Leu Glu Thr Trp Lys Lys Pro	2290	2295	2300
Asp Tyr Glu Pro Pro Val Val His Gly Cys Pro Leu Pro Pro Pro Gln	2305	2310	2315
Ser Pro Pro Val Pro Pro Pro Arg Lys Lys Arg Thr Val Val Leu Thr	2325	2330	2335
Glu Ser Thr Val Ser Ser Ala Leu Ala Glu Leu Ala Thr Lys Ser Phe	2340	2345	2350
Gly Ser Ser Ser Thr Ser Gly Ile Thr Gly Asp Asn Thr Thr Thr Ser	2355	2360	2365
Ser Glu Pro Ala Pro Ser Val Cys Pro Pro Asp Ser Asp Ala Glu Ser	2370	2375	2380
Tyr Ser Ser Met Pro Pro Leu Glu Gly Glu Pro Gly Asp Pro Asp Leu	2385	2390	2395
Ser Asp Gly Ser Trp Ser Thr Val Ser Ser Gly Ala Asp Thr Glu Asp	2405	2410	2415
Val Val Cys Cys Ser Met Ser Tyr Ser Trp Thr Gly Ala Leu Ile Thr	2420	2425	2430
Pro Cys Ala Ala Glu Glu Gln Lys Leu Pro Ile Asn Ala Leu Ser Asn	2435	2440	2445

Ser Leu Leu Arg His His Asn Leu Val Tyr Ser Thr Thr Ser Arg Asn
 2450 2455 2460
 Ala Cys Leu Arg Gln Lys Lys Val Thr Phe Asp Arg Leu Gln Val Leu
 2465 2470 2475 2480
 Asp Asn His Tyr Gln Asp Val Leu Lys Glu Val Lys Ala Ala Ala Ser
 2485 2490 2495
 Lys Val Lys Ala Asn Leu Leu Ser Val Glu Glu Ala Cys Ser Leu Thr
 2500 2505 2510
 Pro Pro His Ser Ala Arg Ser Lys Phe Gly Tyr Gly Ala Lys Asp Val
 2515 2520 2525
 Arg Cys His Ala Arg Lys Ala Val Ser His Ile Asn Ser Val Trp Lys
 2530 2535 2540
 Asp Leu Leu Glu Asp Ser Val Thr Pro Ile Asp Thr Thr Ile Met Ala
 2545 2550 2555 2560
 Lys Asn Glu Val Phe Cys Val Gln Pro Glu Lys Gly Gly Arg Lys Pro
 2565 2570 2575
 Ala Arg Leu Ile Val Phe Pro Asp Leu Gly Val Arg Val Cys Glu Lys
 2580 2585 2590
 Met Ala Leu Tyr Asp Val Val Ser Lys Leu Pro Leu Ala Val Met Gly
 2595 2600 2605
 Ser Ser Tyr Gly Phe Gln Tyr Ser Pro Gly Gln Arg Val Glu Phe Leu
 2610 2615 2620
 Val Gln Ala Trp Lys Ser Lys Lys Thr Pro Met Gly Phe Ser Tyr Asp
 2625 2630 2635 2640
 Thr Arg Cys Phe Asp Ser Thr Val Thr Glu Ser Asp Ile Arg Thr Glu
 2645 2650 2655
 Glu Ala Ile Tyr Gln Cys Cys Asp Leu Asp Pro Gln Ala Arg Val Ala
 2660 2665 2670
 Ile Lys Ser Leu Thr Glu Arg Leu Tyr Val Gly Gly Pro Leu Thr Asn
 2675 2680 2685
 Ser Arg Gly Glu Asn Cys Gly Tyr Arg Arg Cys Arg Ala Ser Gly Val
 2690 2695 2700
 Leu Thr Thr Ser Cys Gly Asn Thr Leu Thr Cys Tyr Ile Lys Ala Arg
 2705 2710 2715 2720
 Ala Ala Cys Arg Ala Ala Gly Leu Gln Asp Cys Thr Met Leu Val Cys
 2725 2730 2735

Gly Asp Asp Leu Val Val Ile Cys Glu Ser Gln Gly Val Gln Glu Asp
 2740 2745 2750
 Ala Ala Ser Leu Arg Ala Phe Thr Glu Ala Met Thr Arg Tyr Ser Ala
 2755 2760 2765
 Pro Pro Gly Asp Pro Pro Gln Pro Glu Tyr Asp Leu Glu Leu Ile Thr
 2770 2775 2780
 Pro Cys Ser Ser Asn Val Ser Val Ala His Asp Gly Ala Gly Lys Arg
 2785 2790 2795 2800
 Val Tyr Tyr Leu Thr Arg Asp Pro Thr Thr Pro Leu Ala Arg Ala Ala
 2805 2810 2815
 Trp Glu Thr Ala Arg His Thr Pro Val Asn Ser Trp Leu Gly Asn Ile
 2820 2825 2830
 Ile Met Phe Ala Pro Thr Leu Trp Ala Arg Met Ile Leu Met Thr His
 2835 2840 2845
 Phe Phe Ser Val Leu Ile Ala Arg Asp Gln Leu Glu Gln Ala Leu Asp
 2850 2855 2860
 Cys Glu Ile Tyr Gly Ala Cys Tyr Ser Ile Glu Pro Leu Asp Leu Pro
 2865 2870 2875 2880
 Pro Ile Ile Gln Arg Leu His Gly Leu Ser Ala Phe Ser Leu His Ser
 2885 2890 2895
 Tyr Ser Pro Gly Glu Ile Asn Arg Val Ala Ala Cys Leu Arg Lys Leu
 2900 2905 2910
 Gly Val Pro Pro Leu Arg Ala Trp Arg His Arg Ala Arg Ser Val Arg
 2915 2920 2925
 Ala Arg Leu Leu Ser Arg Gly Gly Arg Ala Ala Ile Cys Gly Lys Tyr
 2930 2935 2940
 Leu Phe Asn Trp Ala Val Arg Thr Lys Leu Lys Leu Thr Pro Ile Ala
 2945 2950 2955 2960
 Ala Ala Gly Gln Leu Asp Leu Ser Gly Trp Phe Thr Ala Gly Tyr Gly
 2965 2970 2975
 Gly Gly Asp Ile Tyr His Ser Val Ser Arg Ala Arg Pro Arg Trp Phe
 2980 2985 2990
 Trp Phe Cys Leu Leu Leu Leu Ala Ala Gly Val Gly Ile Tyr Leu Leu
 2995 3000 3005
 Pro Asn Arg
 3010

GGC CTG CTG GCT GAA CCC CAG ATT GCC ATG TTC TGT GGC AGA CTG AAC Gly Leu Leu Ala Glu Pro Gln Ile Ala Met Phe Cys Gly Arg Leu Asn 30 35 40	1047
ATG CAC ATG AAT GTC CAG AAT GGG AAG TGG GAT TCA GAT CCA TCA GGG Met His Met Asn Val Gln Asn Gly Lys Trp Asp Ser Asp Pro Ser Gly 45 50 55	1095
ACC AAA ACC TGC ATT GAT ACC AAG GAA ACC CAC GTC ACC GGG GGA AGT Thr Lys Thr Cys Ile Asp Thr Lys Glu Thr His Val Thr Gly Gly Ser 60 65 70	1143
GCC GGC CAC ACC ACG GCT GGG CTT GTT CGT CTC CTT TCA CCA GGC GCC Ala Gly His Thr Thr Ala Gly Leu Val Arg Leu Leu Ser Pro Gly Ala 75 80 85 90	1191
AAG CAG AAC ATC CAA CTG ATC AAC ACC AAC GGC AGT TGG CAC ATC AAT Lys Gln Asn Ile Gln Leu Ile Asn Thr Asn Gly Ser Trp His Ile Asn 95 100 105	1239
AGC ACG GCC TTG AAC TGC AAT GAA AGC CTT AAC ACC GGC TGG TTA GCA Ser Thr Ala Leu Asn Cys Asn Glu Ser Leu Asn Thr Gly Trp Leu Ala 110 115 120	1287
GGG CTC TTC TAT CAC CAC AAA TTC AAC TCT TCA GGT TGT CCT GAG AGG Gly Leu Phe Tyr His His Lys Phe Asn Ser Ser Gly Cys Pro Glu Arg 125 130 135	1335
TTG GCC AGC TGC CGA CGC CTT ACC GAT TTT GCC CAG GGC GGG GGT CCT Leu Ala Ser Cys Arg Arg Leu Thr Asp Phe Ala Gln Gly Gly Gly Pro 140 145 150	1383
ATC AGT TAC GCC AAC GGA AGC GGC CTC GAT GAA CGC CCC TAC TGC TGG Ile Ser Tyr Ala Asn Gly Ser Gly Leu Asp Glu Arg Pro Tyr Cys Trp 155 160 165 170	1431
CAC TAC CCT CCA AGA CCT TGT GGC ATT GTG CCC GCA AAG AGC GTG TGT His Tyr Pro Pro Arg Pro Cys Gly Ile Val Pro Ala Lys Ser Val Cys 175 180 185	1479
GGC CCG GTA TAT TGC TTC ACT CCC AGC CCC GTG GTG GTG GGA ACG ACC Gly Pro Val Tyr Cys Phe Thr Pro Ser Pro Val Val Val Gly Thr Thr 190 195 200	1527
GAC AGG TCG GGC GCG CCT ACC TAC AGC TGG GGT GCA AAT GAT ACG GAT Asp Arg Ser Gly Ala Pro Thr Tyr Ser Trp Gly Ala Asn Asp Thr Asp 205 210 215	1575
GTC TTT GTC CTT AAC AAC ACC AGG CCA CCG CTG GGC AAT TGG TTC GGT Val Phe Val Leu Asn Asn Thr Arg Pro Pro Leu Gly Asn Trp Phe Gly 220 225 230	1623
TGC ACC TGG ATG AAC TCA ACT GGA TTC ACC AAA GTG TGC GGA GCG CCC Cys Thr Trp Met Asn Ser Thr Gly Phe Thr Lys Val Cys Gly Ala Pro 235 240 245 250	1671

CCT TGT GTC ATC GGA GGG GTG GGC AAC AAC ACC TTG CTC TGC CCC ACT	1719
Pro Cys Val Ile Gly Gly Val Gly Asn Asn Thr Leu Leu Cys Pro Thr	
255 260 265	
GAT TGC TTC CGC AAG CAT CCG GAA GCC ACA TAC TCT CGG TGC GGC TCC	1767
Asp Cys Phe Arg Lys His Pro Glu Ala Thr Tyr Ser Arg Cys Gly Ser	
270 275 280	
GGT CCC TGG ATT ACA CCC AGG TGC ATG GTC GAC TAC CCG TAT AGG CTT	1815
Gly Pro Trp Ile Thr Pro Arg Cys Met Val Asp Tyr Pro Tyr Arg Leu	
285 290 295	
TGG CAC TAT CCT TGT ACC ATC AAT TAC ACC ATA TTC AAA GTC AGG ATG	1863
Trp His Tyr Pro Cys Thr Ile Asn Tyr Thr Ile Phe Lys Val Arg Met	
300 305 310	
TAC GTG GGA GGG GTC GAG CAC AGG CTG GAA GCG GCC TGC AAC TGG ACG	1911
Tyr Val Gly Gly Val Glu His Arg Leu Glu Ala Ala Cys Asn Trp Thr	
315 320 325 330	
CGG GGC GAA CGC TGT GAT CTG GAA GAC AGG GAC AGG TCC GAG CTC AGC	1959
Arg Gly Glu Arg Cys Asp Leu Glu Asp Arg Asp Arg Ser Glu Leu Ser	
335 340 345	
CCG TTA CTG CTG TCC ACC ACG CAG TGG CAG GTC CTT CCG TGT TCT TTC	2007
Pro Leu Leu Leu Ser Thr Thr Gln Trp Gln Val Leu Pro Cys Ser Phe	
350 355 360	
ACG ACC CTG CCA GCC TTG TCC ACC GGC CTC ATC CAC CTC CAC CAG AAC	2055
Thr Thr Leu Pro Ala Leu Ser Thr Gly Leu Ile His Leu His Gln Asn	
365 370 375	
ATT GTG GAC GTG CAG TAC TTG TAC GGG GTA GGG TCA AGC ATC GCG TCC	2103
Ile Val Asp Val Gln Tyr Leu Tyr Gly Val Gly Ser Ser Ile Ala Ser	
380 385 390	
TGG GCT ATT AAG TGG GAG TAC GAC GTT CTC CTG TTC CTT CTG CTT GCA	2151
Trp Ala Ile Lys Trp Glu Tyr Asp Val Leu Leu Phe Leu Leu Leu Ala	
395 400 405 410	
GAC GCG CGC GTT TGC TCC TGC TTG TGG ATG ATG TTA CTC ATA TCC CAA	2199
Asp Ala Arg Val Cys Ser Cys Leu Trp Met Met Leu Leu Ile Ser Gln	
415 420 425	
GCG GAG GCG GCT TTG GAG ATC TCT GAA GTG AAG ATG GAT GCA GAA TTC	2247
Ala Glu Ala Ala Leu Glu Ile Ser Glu Val Lys Met Asp Ala Glu Phe	
430 435 440	
CGA CAT GAC TCA GGA TAT GAA GTT CAT CAT CAA AAA TTG GTG TTC TTT	2295
Arg His Asp Ser Gly Tyr Glu Val His His Gln Lys Leu Val Phe Phe	
445 450 455	
GCA GAA GAT GTG GGT TCA AAC AAA GGT GCA ATC ATT GGA CTC ATG GTG	2343
Ala Glu Asp Val Gly Ser Asn Lys Gly Ala Ile Ile Gly Leu Met Val	

460	465	470	
GGC GGT GTT GTC ATA GCG ACA GTG ATC GTC ATC ACC TTG GTG ATG CTG Gly Gly Val Val Ile Ala Thr Val Ile Val Ile Thr Leu Val Met Leu 475 480 485 490			2391
AAG AAG AAA CAG TAC ACA TCC ATT CAT CAT GGT GTG GTG GAG GTT GAC Lys Lys Lys Gln Tyr Thr Ser Ile His His Gly Val Val Glu Val Asp 495 500 505			2439
GCC GCT GTC ACC CCA GAG GAG CGC CAC CTG TCC AAG ATG CAG CAG AAC Ala Ala Val Thr Pro Glu Glu Arg His Leu Ser Lys Met Gln Gln Asn 510 515 520			2487
GGC TAC GAA AAT CCA ACC TAC AAG TTC TTT GAG CAG ATG CAG AAC Gly Tyr Glu Asn Pro Thr Tyr Lys Phe Phe Glu Gln Met Gln Asn 525 530 535			2532
TAGACCCCCG CCACAGCAGC CTCTGAAGTT GGACAGCAAA ACCATTGCTT CACTACCCAT			2592
CGGTGTCCAT TTATAGAATA ATGTGGGAAG AAACAAACCC GTTTTATGAT TTA CTCTATTA			2652
TGCGCTTTTG ACAGCTGTGC TGTAACACAA GTAGATGCCT GAACTTGAAT TAATCCACAC			2712
ATCAGTATTG TATTCTATCT CTCTTTACAT TTTGGTCTCT ATACTACATT ATTAATGGGT			2772
TTTGTGTACT GTAAAGAATT TAGCTGTATC AACTAGTGC ATGAATAGGC CGCTCGAGCA			2832
TGCATCTAGA GGGCCCTATT CTATAGTGTC ACCTAAATGC TCGCTGATCA GCCTCGACTG			2892
TGCCTTCTAG TTGCCAGCCA TCTGTTGTTT GCCCCTCCCC CGTGCCTTCC TTGACCCTGG			2952
AAGGTGCCAC TCCCACTGTC CTTTCCTAAT AAAATGAGGA AATTGCATCG CATTGTCTGA			3012
GTAGGTGTCA TTCTATTCTG GGGGGTGGGG TGGGGCAGGA CAGCAAGGGG GAGGATTGGG			3072
AAGACAATAG CAGGCATGCT GGGGATGCGG TGGGCTCTAT GGAACAGCT GGGGCTCGAG			3132
GGGGGATCCC CACGCGCCCT GTAGCGGCGC ATTAAGCGCG GCGGGTGTGG TGGTTACGCG			3192
CAGCGTGACC GCTACACTTG CCAGCGCCCT AGCGCCCGCT CCTTTCGCTT TCTTCCCTTC			3252
CTTTCGCGC ACGTTCGCGG GCTTTCCCCG TCAAGCTCTA AATCGGGGCA TCCCTTTAGG			3312
GTTCGATTT AGTGCTTTAC GGCACCTCGA CCCCCAAAAA CTTGATTAGG GTGATGGTTC			3372
ACGTAGTGGG CCATCGCCCT GATAGACGGT TTTTCGCCTT TACTGAGCAC TCTTTAATAG			3432
TGGACTCTTG TTCCAAACTG GAACAACACT CAACCCTATC TCGGTCTATT CTTTTGATTT			3492
ATAAGATTT CATCGCCATG TAAAAGTGT ACAATTAGCA TTAAATTACT TCTTTATATG			3552
CTACTATTCT TTTGGCTTCG TTCACGGGGT GGGTACCGAG CTCGAATTCT GTGGAATGTG			3612
TGTCAGTTAG GGTGTGGAAA GTCCCCAGGC TCCCCAGGCA GGCAGAAGTA TGCAAAGCAT			3672

GCATCTCAAT TAGTCAGCAA CCAGGTGTGG AAAGTCCCCA GGCTCCCCAG CAGGCAGAAG 3732
TATGCAAAGC ATGCATCTCA AAGC AACCATAGTC CCGCCCCCTAA CTCCGCCCCAT 3792
CCCGCCCCCTA ACTCCGCCCCA GTTCCGCCCCA TTCTCCGCCC CATGGCTGAC TAATTTTTTTT 3852
TATTTATGCA GAGGCCGAGG CCGCCTCGGC CTCTGAGCTA TTCCAGAAGT AGTGAGGAGG 3912
CTTTTTTGA GGCCTAGGCT TTTGCAAAAA GCTCCCGGGA GCTTGGATAT CCATTTTCGG 3972
ATCTGATCAA GAGACAGGAT GAGGATCGTT TCGCATGATT GAACAAGATG GATTGCACGC 4032
AGGTTCTCCG GCCGCTTGGG TGGAGAGGCT ATTCCGGCTAT GACTGGGCAC AACAGACAAT 4092
CGGCTGCTCT GATGCCGCCG TGTTCGGCT GTCAGCGCAG GGGCGCCCGG TTCTTTTTTGT 4152
CAAGACCGAC CTGTCCGGTG CCCTGAATGA ACTGCAGGAC GAGGCAGCGC GGCTATCGTG 4212
GCTGGCCACG ACGGGCGTTC CTTGCGCAGC TGTGCTCGAC GTTGTCACCTG AAGCGGGAAG 4272
GGACTGGCTG CTATTGGGCG AAGTGCCGGG GCAGGATCTC CTGTCATCTC ACCTTGCTCC 4332
TGCCGAGAAA GTATCCATCA TGGCTGATGC AATGCGGCGG CTGCATACGC TTGATCCGGC 4392
TACCTGCCCCA TCGACCACC AAGCGAAACA TCGCATCGAG CGAGCACGTA CTCGGATGGA 4452
AGCCGGTCTT GTCGATCAGG ATGATCTGGA CGAAGAGCAT CAGGGGCTCG CGCCAGCCGA 4512
ACTGTTCCGC AGGCTCAAGG CGCGCATGCC CGACGGCGAG GATCTCGTCG TGACCCATGG 4572
CGATGCCTGC TTGCCGAATA TCATGGTGGA AAATGGCCGC TTTTCTGGAT TCATCGACTG 4632
TGGCCGGCTG GGTGTGGCGG ACCGCTATCA GGACATAGCG TTGGCTACCC GTGATATTGC 4692
TGAAGAGCTT GGCGGCGAAT GGGCTGACCG CTTCTCTGTG CTTTACGGTA TCGCCGCTCC 4752
CGATTGCGAG CGCATCGCCT TCTATCGCCT TCTTGACGAG TTCTTCTGAG CGGGACTCTG 4812
GGGTTGCAAA TGACCGACCA AGCGACGCC AACCTGCCAT CACGAGATTT CGATTCCACC 4872
GCCGCCTTCT ATGAAAGGTT GGGCTTCGGA ATCGTTTTCC GGGACGCCGG CTGGATGATC 4932
CTCCAGCGCG GGGATCTCAT GCTGGAGTTC TTCGCCCACC CCAACTTGTT TATTGCAGCT 4992
TATAATGGTT ACAAATAAAG CAATAGCATC ACAAATTTCA CAAATAAAGC ATTTTTTTTCA 5052
CTGCATTCTA GTTGTGGTTT GTCCAAACTC ATCAATGTAT CTTATCATGT CTGGATCCCG 5112
TCGACCTCGA GAGCTTGGCG TAATCATGGT CATAGCTGTT TCCTGTGTGA AATTGTTATC 5172
CGCTCACAAT TCCACACAAC ATACGAGCCG GAAGCATAAA GTGTAAAGCC TGGGGTGCCT 5232
AATGAGTGAG CTAATCACA TTAATTGCGT TGCCTCACT GCCCGCTTTC CAGTCGGGAA 5292

ACCTGTCGTG CCAGCTGCAT TAATGAATCG GCCAACGCGC GGGGAGAGGC GGTTCGCGTA 5352
TTGGGCGCTC TTCCGCTTCC TCGCTCACTG ACTCGCTGCG CTCGGTCGTT CGGCTGCGGC 5412
GAGCGGTATC AGCTCACTCA AAGGCGGTAA TACGGTTATC CACAGAATCA GGGGATAACG 5472
CAGGAAAGAA CATGTGAGCA AAAGGCCAGC AAAAGGCCAG GAACCGTAAA AAGGCCGCGT 5532
TGCTGGCGTT TTTCCATAGG CTCCGCCCCC CTGACGAGCA TCACAAAAAT CGACGCTCAA 5592
GTCAGAGGTG GCGAAACCCG ACAGGACTAT AAAGATACCA GGC GTTTCCC CCTGGAAGCT 5652
CCCTCGTGCG CTCTCCTGTT CCGACCCTGC CGCTTACCGG ATACCTGTCC GCCTTTCTCC 5712
CTTCGGGAAG CGTGGCGCTT TCTCAATGCT CACGCTGTAG GTATCTCAGT TCGGTGTAGG 5772
TCGTTGCTC CAAGCTGGGC TGTGTGCACG AACCCCCGT TCAGCCCGAC CGCTGCGCCT 5832
TATCCGGTAA CTATCGTCTT GAGTCCAACC CGGTAAGACA CGACTTATCG CCACTGGCAG 5892
CAGCCACTGG TAACAGGATT AGCAGAGCGA GGTATGTAGG CGGTGCTACA GAGTTCTTGA 5952
AGTGGTGGCC TAACTACGGC TACACTAGAA GGACAGTATT TGGTATCTGC GCTCTGCTGA 6012
AGCCAGTTAC CTTCGGAAAA AGAGTTGGTA GCTCTTGATC CGGCAAACAA ACCACCGCTG 6072
GTAGCGGTGG TTTTTTTGTT TGCAAGCAGC AGATTACGCG CAGAAAAAAA GGATCTCAAG 6132
AAGATCCTTT GATCTTTTCT ACGGGGTCTG ACGCTCAGTG GAACGAAAAC TCACGTTAAG 6192
GGATTTTGGT CATGAGATTA TCAAAAAGGA TCTTCACCTA GATCCTTTTA AATTAAAAAT 6252
GAAGTTTAA ATCAATCTAA AGTATATATG AGTAAACTTG GTCTGACAGT TACCAATGCT 6312
TAATCAGTGA GGCACCTATC TCAGCGATCT GTCTATTTG TTCATCCATA GTTGCCCTGAC 6372
TCCCCGTCGT GTAGATAACT ACGATACGGG AGGGCTTACC ATCTGGCCCC AGTGCTGCAA 6432
TGATACCGCG AGACCCACGC TCACCGGCTC CAGATTTATC AGCAATAAAC CAGCCAGCCG 6492
GAAGGGCCGA GCGCAGAAGT GGTCTGCAA CTTTATCCGC CTCCATCCAG TCTATTAATT 6552
GTTGCCGGGA AGCTAGAGTA AGTAGTTGCG CAGTTAATAG TTGCGCAAC GTTGTTGCCA 6612
TTGCTACAGG CATCGTGGTG TCACGCTCGT CGTTTGGTAT GGCTTCATTC AGCTCCGGTT 6672
CCCAACGATC AAGGCGAGTT ACATGATCCC CCATGTTGTG CAAAAAAGCG GTTAGCTCCT 6732
TCGGTCCTCC GATCGTTGTC AGAAGTAAGT TGGCCGAGT GTTATCACTC ATGGTTATGG 6792
CAGCACTGCA TAATTCTCTT ACTGTCATGC CATCCGTAAG ATGCTTTTCT GTGACTGGTG 6852
AGTACTCAAC CAAGTCATTC TGAGAATAGT GTATGCGGCG ACCGAGTTGC TCTTGCCCCG 6912
CGTCAATACG GGATAATACC GCGCCACATA GCAGAACTTT AAAAGTGCTC ATCATTGGAA 6972

AACGTTCTTC GGGGCGAAAA CTCTCAAGGA TCTTACCGCT GTTGAGATCC AGTTCGATGT 7032
 AACCCACTCG TGCACCCAAC TGATCTTCAG CATCTTTTAC TTTCACCAGC GTTCTGCGGT 7092
 GAGCAAAAAC AGGAAGGCAA AATGCCGCAA AAAAGGGAAT AAGGGCGACA CGGAAATGTT 7152
 GAATACTCAT ACTCTTCCTT TTTCAATATT ATTGAAGCAT TTATCAGGGT TATTGTCTCA 7212
 TGAGCGGATA CATATTTGAA TGTATTTAGA AAAATAAACA AATAGGGGTT CCGCGCACAT 7272
 TTCCCCGAAA AGTGCCACCT GACGTC 7298

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 537 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Met Leu Pro Gly Leu Ala Leu Leu Leu Leu Ala Ala Trp Thr Ala Arg
 1 5 10 15
 Ala Leu Glu Val Pro Thr Asp Gly Asn Ala Gly Leu Leu Ala Glu Pro
 20 25 30
 Gln Ile Ala Met Phe Cys Gly Arg Leu Asn Met His Met Asn Val Gln
 35 40 45
 Asn Gly Lys Trp Asp Ser Asp Pro Ser Gly Thr Lys Thr Cys Ile Asp
 50 55 60
 Thr Lys Glu Thr His Val Thr Gly Gly Ser Ala Gly His Thr Thr Ala
 65 70 75 80
 Gly Leu Val Arg Leu Leu Ser Pro Gly Ala Lys Gln Asn Ile Gln Leu
 85 90 95
 Ile Asn Thr Asn Gly Ser Trp His Ile Asn Ser Thr Ala Leu Asn Cys
 100 105 110
 Asn Glu Ser Leu Asn Thr Gly Trp Leu Ala Gly Leu Phe Tyr His His
 115 120 125
 Lys Phe Asn Ser Ser Gly Cys Pro Glu Arg Leu Ala Ser Cys Arg Arg
 130 135 140
 Leu Thr Asp Phe Ala Gln Gly Gly Gly Pro Ile Ser Tyr Ala Asn Gly
 145 150 155 160

57

Ser Gly Leu Asp Glu Arg Pro Tyr Cys Trp His Tyr Pro Pro Arg Pro
 165 170 175

Cys Gly Ile Val Pro Ala Lys Ser Val Cys Gly Pro Val Tyr Cys Phe
 180 185 190

Thr Pro Ser Pro Val Val Val Gly Thr Thr Asp Arg Ser Gly Ala Pro
 195 200 205

Thr Tyr Ser Trp Gly Ala Asn Asp Thr Asp Val Phe Val Leu Asn Asn
 210 215 220

Thr Arg Pro Pro Leu Gly Asn Trp Phe Gly Cys Thr Trp Met Asn Ser
 225 230 235 240

Thr Gly Phe Thr Lys Val Cys Gly Ala Pro Pro Cys Val Ile Gly Gly
 245 250 255

Val Gly Asn Asn Thr Leu Leu Cys Pro Thr Asp Cys Phe Arg Lys His
 260 265 270

Pro Glu Ala Thr Tyr Ser Arg Cys Gly Ser Gly Pro Trp Ile Thr Pro
 275 280 285

Arg Cys Met Val Asp Tyr Pro Tyr Arg Leu Trp His Tyr Pro Cys Thr
 290 295 300

Ile Asn Tyr Thr Ile Phe Lys Val Arg Met Tyr Val Gly Gly Val Glu
 305 310 315 320

His Arg Leu Glu Ala Ala Cys Asn Trp Thr Arg Gly Glu Arg Cys Asp
 325 330 335

Leu Glu Asp Arg Asp Arg Ser Glu Leu Ser Pro Leu Leu Leu Ser Thr
 340 345 350

Thr Gln Trp Gln Val Leu Pro Cys Ser Phe Thr Thr Leu Pro Ala Leu
 355 360 365

Ser Thr Gly Leu Ile His Leu His Gln Asn Ile Val Asp Val Gln Tyr
 370 375 380

Leu Tyr Gly Val Gly Ser Ser Ile Ala Ser Trp Ala Ile Lys Trp Glu
 385 390 395 400

Tyr Asp Val Leu Leu Phe Leu Leu Leu Ala Asp Ala Arg Val Cys Ser
 405 410 415

Cys Leu Trp Met Met Leu Leu Ile Ser Gln Ala Glu Ala Ala Leu Glu
 420 425 430

Ile Ser Glu Val Lys Met Asp Ala Glu Phe Arg His Asp Ser Gly Tyr
 435 440 445

Glu Val His His Gln Lys Leu Val Phe Phe Ala Glu Asp Val Gly Ser

58

450	455	460
Asn Lys Gly Ala Ile Ile Gly Leu Met Val Gly Gly Val Val Ile Ala		
465	470	475 480
Thr Val Ile Val Ile Thr Leu Val Met Leu Lys Lys Lys Gln Tyr Thr		
	485	490 495
Ser Ile His His Gly Val Val Glu Val Asp Ala Ala Val Thr Pro Glu		
	500	505 510
Glu Arg His Leu Ser Lys Met Gln Gln Asn Gly Tyr Glu Asn Pro Thr		
	515	520 525
Tyr Lys Phe Phe Glu Gln Met Gln Asn		
	530	535

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 7106 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: circular

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 922..2022

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

GACGGATCGG GAGATCTCCC GATCCCCTAT GGTGACTCT CAGTACAATC TGCTCTGATG	60
CCGCATAGTT AAGCCAGTAT CTGCTCCCTG CTTGTGTGTT GGAGGTCGCT GAGTAGTGCG	120
CGAGCAAAAT TTAAGCTACA ACAAGGCAAG GCTTGACCGA CAATTGCATG AAGAATCTGC	180
TTAGGGTTAG GCGTTTGTGCG CTGCTTCGCG ATGTACGGGC CAGATATACG CGTTGACATT	240
GATTATTGAC TAGTTATTAA TAGTAATCAA TTACGGGGTC ATTAGTTCAT AGCCCATATA	300
TGGAGTTCCG CGTTACATAA CTTACGGTAA ATGGCCCGCC TGGCTGACCG CCCAACGACC	360
CCCGCCCCATT GACGTCAATA ATGACGTATG TTCCCATAGT AACGCCAATA GGGACTTTCC	420
ATTGACGTCA ATGGGTGGAC TATTTACGGT AAAGTGCCCA CTTGGCAGTA CATCAAGTGT	480
ATCATATGCC AAGTACGCC CCTATTGACG TCAATGACGG TAAATGGCCC GCCTGGCATT	540
ATGCCCAGTA CATGACCTTA TGGGACTTTC CTAATTGGCA GTACATCTAC GTATTAGTCA	600

TCGCTATTAC CATGGTGATG CGGTTTTGGC AGTACATCAA TGGGCGTGGA TAGCGGTTTG	660
ACTCACGGGG ATTTCCAAGT CTCCACCCCA TTGACGTCAA TGGGAGTTTG TTTTGGCACC	720
AAAATCAACG GGACTTTCCA AAATGTCGTA ACAACTCCGC CCCATTGACG CAAATGGGCG	780
GTAGGCGTGT ACGGTGGGAG GTCTATATAA GCAGAGCTCT CTGGCTAACT AGAGAACCCA	840
CTGCTTAACT GGCTTATCGA AATTAATACG ACTCACTATA GGGAGACCGG AAGCTTTGCT	900
CTAGACTGGA ATTCGGGGCG G ATG CTG CCC GGT TTG GCA CTG CTC CTG CTG	951
Met Leu Pro Gly Leu Ala Leu Leu Leu Leu	
1 5 10	
GCC GCC TGG ACG GCT CGG GCG CTG GAG GTA CCC ACT GAT GGT AAT GCT	999
Ala Ala Trp Thr Ala Arg Ala Leu Glu Val Pro Thr Asp Gly Asn Ala	
15 20 25	
GGC CTG CTG GCT GAA CCC CAG ATT GCC ATG TTC TGT GGC AGA CTG AAC	1047
Gly Leu Leu Ala Glu Pro Gln Ile Ala Met Phe Cys Gly Arg Leu Asn	
30 35 40	
ATG CAC ATG AAT GTC CAG AAT GGG AAG TGG GAT TCA GAT CCA TCA GGG	1095
Met His Met Asn Val Gln Asn Gly Lys Trp Asp Ser Asp Pro Ser Gly	
45 50 55	
ACC AAA ACC TGC ATT GAT ACC AAG GAA ACC CAC GTC ACC GGG GGA AGT	1143
Thr Lys Thr Cys Ile Asp Thr Lys Glu Thr His Val Thr Gly Gly Ser	
60 65 70	
GCC GGC CAC ACC ACG GCT GGG CTT GTT CGT CTC CTT TCA CCA GGC GCC	1191
Ala Gly His Thr Thr Ala Gly Leu Val Arg Leu Leu Ser Pro Gly Ala	
75 80 85 90	
AAG CAG AAC ATC CAA CTG ATC AAC ACC AAC GGC AGT TGG CAC ATC AAT	1239
Lys Gln Asn Ile Gln Leu Ile Asn Thr Asn Gly Ser Trp His Ile Asn	
95 100 105	
AGC ACG GCC TTG AAC TGC AAT GAA AGC CTT AAC ACC GGC TGG TTA GCA	1287
Ser Thr Ala Leu Asn Cys Asn Glu Ser Leu Asn Thr Gly Trp Leu Ala	
110 115 120	
GGG CTC TTC TAT CAC CAC AAA TTC AAC TCT TCA GGT TGT CCT GAG AGG	1335
Gly Leu Phe Tyr His His Lys Phe Asn Ser Ser Gly Cys Pro Glu Arg	
125 130 135	
TTG GCC AGC TGC CGA CGC CTT ACC GAT TTT GCC CAG GGC GGG GGT CCT	1383
Leu Ala Ser Cys Arg Arg Leu Thr Asp Phe Ala Gln Gly Gly Gly Pro	
140 145 150	
ATC AGT TAC GCC AAC GGA AGC GGC CTC GAT GAA CGC CCC TAC TGC TGG	1431
Ile Ser Tyr Ala Asn Gly Ser Gly Leu Asp Glu Arg Pro Tyr Cys Trp	
155 160 165 170	
CAC TAC CCT CCA AGA CCT TGT GGC ATT GTG CCC GCA AAG AGC GTG TGT	1479



60

[illegible]

TCACCTTGGT GATGCTGAAG AAGAAACAGT ACACATCCAT TCATCATGGT GTGGTGGAGG	2242
TTGACGCCGC TGTCACCCCA GAGGAGCGCC ACCTGTCCAA GATGCAGCAG AACGGCTACG	2302
AAAATCCAAC CTACAAGTTC TTTGAGCAGA TGCAGAACTA GACCCCCGCC ACAGCAGCCT	2362
CTGAAGTTGG ACAGCAAAAC CATTGCTTCA CTACCCATCG GTGTCCATTT ATAGAATAAT	2422
GTGGGAAGAA ACAAACCCGT TTTATGATTT ACTCATTATC GCCTTTTGAC AGCTGTGCTG	2482
TAACACAAGT AGATGCCTGA ACTTGAATTA ATCCACACAT CAGTAATGTA TTCTATCTCT	2542
CTTTACATTT TGGTCTCTAT ACTACATTAT TAATGGGTTT TGTGTACTGT AAAGAATTTA	2602
GCTGTATCAA ACTAGTGCAT GAATAGGCCG CTCGAGCATG CATCTAGAGG GCCCTATTCT	2662
ATAGTGTAC CTAAATGCTC GCTGATCAGC CTCGACTGTG CCTTCTAGTT GCCAGCCATC	2722
TGTTGTTTGC CCCTCCCCCG TGCCTTCCTT GACCCTGGAA GGTGCCACTC CCACTGTCTT	2782
TTCTAATAA AATGAGGAAA TTGCATCGCA TTGTCTGAGT AGGTGTCATT CTATTCTGGG	2842
GGGTGGGGTG GGGCAGGACA GCAAGGGGGA GGATTGGGAA GACAATAGCA GGCATGCTGG	2902
GGATGCGGTG GGCTCTATGG AACCAGCTGG GGCTCGAGGG GGGATCCCCA CGCGCCCTGT	2962
AGCGGCGCAT TAAGCGCGGC GGGTGTGGTG GTTACGCGCA GCGTGACCGC TACACTTGCC	3022
AGCGCCCTAG CGCCCGCTCC TTTCGCTTTC TTCCCTTCCT TTCTCGCCAC GTTCGCCGGC	3082
TTCCCCGTC AAGCTCTAAA TCGGGGCATC CCTTTAGGGT TCCGATTTAG TGCTTTACGG	3142
CACCTCGACC CCAAAAACT TGATTAGGGT GATGGTTCAC GTAGTGGGCC ATCGCCCTGA	3202
TAGACGGTTT TTCGCCTTTA CTGAGCACTC TTTAATAGTG GACTCTTGTT CCAAAGTGA	3262
ACAACACTCA ACCCTATCTC GGTCTATTCT TTTGATTTAT AAGATTTCCA TCGCCATGTA	3322
AAAGTGTTAC AATTAGCATT AAATTACTTC TTTATATGCT ACTATTCTTT TGGCTTCGTT	3382
CACGGGGTGG GTACCGAGCT CGAATTCTGT GGAATGTGTG TCAGTTAGGG TGTGGAAAGT	3442
CCCCAGGCTC CCCAGGCAGG CAGAAGTATG CAAAGCATGC ATCTCAATTA GTCAGCAACC	3502
AGGTGTGGAA AGTCCCCAGG CTCCCCAGCA GGCAGAAGTA TGCAAAGCAT GCATCTCAAT	3562
TAGTCAGCAA CCATAGTCCC GCCCCTAACT CCGCCCATCC CGCCCCCTAAC TCCGCCAGT	3622
TCCGCCCAT CTCCGCCCCA TGGCTGACTA ATTTTTTTTA TTTATGCAGA GGCCGAGGCC	3682
GCCTCGGCCT CTGAGCTATT CCAGAAGTAG TGAGGAGGCT TTTTGGAGG CCTAGGCTTT	3742
TGCAAAAAGC TCCCGGGAGC TTGGATATCC ATTTTCGGAT CTGATCAAGA GACAGGATGA	3802
GGATCGTTTC GCATGATTGA ACAAGATGGA TTGCACGCAG GTTCTCCGGC CGCTTGGGTG	3862

GAGAGGCTAT TCGGCTATGA CTGGGCACAA CAGACAATCG GCTGCTCTGA TGCCGCCGTG 3922
TTCCGGCTGT CAGCGCAGGG GCGCCCGGTT CTTTTGTCA AGACCGACCT GTCCGGTGCC 3982
CTGAATGAAC TGCAGGACGA GGCAGCGCGG CTATCGTGCC TGGCCACGAC GGGCGTTCCT 4042
TGCGCAGCTG TGCTCGACGT TGTCCTGAA GCGGGAAGGG ACTGGCTGCT ATTGGGCGAA 4102
GTGCCGGGGC AGGATCTCCT GTCATCTCAC CTTGCTCCTG CCGAGAAAGT ATCCATCATG 4162
GCTGATGCAA TGCGGCGGCT GCATACGCTT GATCCGGCTA CCTGCCCATT CGACCACCAA 4222
GCGAAACATC GCATCGAGCG AGCACGTA CTGATGGAAG CCGGTCTTGT CGATCAGGAT 4282
GATCTGGACG AAGAGCATCA GGGGCTCGCG CCAGCCGAAC TGTTGCCAG GCTCAAGGCG 4342
CGCATGCCCC ACGGCGAGGA TCTCGTCGTG ACCCATGGCG ATGCCTGCTT GCCGAATATC 4402
ATGGTGAAA ATGCGCGCTT TTCTGGATT ATCGACTGTG GCCGGCTGGG TGTGGCGGAC 4462
CGCTATCAGG ACATAGCGTT GGCTACCCGT GATATTGCTG AAGAGCTTGG CGGCGAATGG 4522
GCTGACCGCT TCCTCGTGCT TTACGGTATC GCCGCTCCCG ATTCGCAGCG CATCGCCTTC 4582
TATCGCCTTC TTGACGAGTT CTTCTGAGCG GGACTCTGGG GTTCGAAATG ACCGACCAAG 4642
CGACGCCCAA CCTGCCATCA CGAGATTTCG ATTCCACCGC CGCCTTCTAT GAAAGGTTGG 4702
GCTTCGGAAT CGTTTTCCGG GACGCCGGCT GGATGATCCT CCAGCGCGGG GATCTCATGC 4762
TGGAGTTCTT CGCCACCCC AACTTGTTTA TTGCAGCTTA TAATGGTTAC AAATAAGCA 4822
ATAGCATCAC AAATTTTACA AATAAAGCAT TTTTTCCTT GCATTCTAGT TGTGGTTTGT 4882
CCAAACTCAT CAATGTATCT TATCATGTCT GGATCCCGTC GACCTCGAGA GCTTGGCGTA 4942
ATCATGGTCA TAGCTGTTTC CTGTGTGAAA TTGTATCCG CTCACAATC CACACAACAT 5002
ACGAGCCGGA AGCATAAAGT GTAAAGCCTG GGGTGCCTAA TGAGTGAGCT AACTCACATT 5062
AATGCGTTG CGCTCACTGC CCGCTTTCCA GTCGGGAAAC CTGTCGTGCC AGCTGCATTA 5122
ATGAATCGGC CAACGCGCGG GGAGAGGCGG TTTGCGTATT GGGCGCTCTT CCGCTTCCTC 5182
GCTCACTGAC TCGCTGCGCT CGGTCTTCG GCTGCGGCGA GCGGTATCAG CTCACTCAA 5242
GGCGGTAATA CGGTTATCCA CAGAATCAGG GGATAACGCA GGAAAGAACA TGTGAGCAA 5302
AGGCCAGCAA AAGGCCAGGA ACCGTAAAAA GGCCGCGTTG CTGGCGTTT TCCATAGGCT 5362
CCGCCCCCCT GACGAGCATC AAAAAATCG ACGCTCAAGT CAGAGGTGGC GAAACCCGAC 5422
AGGACTATAA AGATACCAGG CGTTTCCCC TGGAAGCTCC CTCGTGCGCT CTCCTGTTCC 5482

GACCCTGCCG CTTACCGGAT ACCTGTCCGC CTTTCTCCCT TCGGGAAGCG TGGCGCTTTC 5542
TCAATGCTCA CGCTGTAGGT ATCTCAGTTC GGTGTAGGTC GTTCGCTCCA AGCTGGGCTG 5602
TGTGCACGAA CCCCCCGTTC AGCCCGACCG CTGCGCCTTA TCCGGTAACT ATCGTCTTGA 5662
GTCCAACCCG GTAAGACACG ACTTATCGCC ACTGGCAGCA GCCACTGGTA ACAGGATTAG 5722
CAGAGCGAGG TATGTAGGCG GTGCTACAGA GTTCTTGAAG TGGTGGCCTA ACTACGGCTA 5782
CACTAGAAGG ACAGTATTTG GSTATCTGCGC TCTGCTGAAG CCAGTTACCT TCGGAAAAAG 5842
AGTTGGTAGC TCTTGATCCG GCAAACAAAC CACCGCTGGT AGCGGTGGTT TTTTGTGTTG 5902
CAAGCAGCAG ATTACGCGCA GAAAAAAGG ATCTCAAGAA GATCCTTTGA TCTTTTCTAC 5962
GGGGTCTGAC GCTCAGTGGA ACGAAAACTC ACGTTAAGGG ATTTTGGTCA TGAGATTATC 6022
AAAAAGGATC TTCACCTAGA TCCTTTTAAA TTAAAAATGA AGTTTAAAT CAATCTAAAG 6082
TATATATGAG TAAACTTGGT CTGACAGTTA CCAATGCTTA ATCAGTGAGG CACCTATCTC 6142
AGCGATCTGT CTATTTCTGT CATCCATAGT TGCCTGACTC CCCGTCGTGT AGATAACTAC 6202
GATACGGGAG GGCTTACCAT CTGGCCCCAG TGCTGCAATG ATACCGCGAG ACCCAGGCTC 6262
ACCGGCTCCA GATTTATCAG CAATAAACCA GCCAGCCGGA AGGGCCGAGC GCAGAAAGTGG 6322
TCCTGCAACT TTATCCGCCT CCATCCAGTC TATTAATTGT TGCCGGGAAG CTAGAGTAAG 6382
TAGTTCGCCA GTTAATAGTT TGCGCAACGT TGTTGCCATT GCTACAGGCA TCGTGGTGTG 6442
ACGCTCGTCG TTTGGTATGG CTTCAATCAG CTCCGGTTCC CAACGATCAA GGCGAGTTAC 6502
ATGATCCCC ATGTTGTGCA AAAAAGCGGT TAGCTCCTTC GGTCTCCGA TCGTTGTCAG 6562
AAGTAAGTTG GCCGCAGTGT TATCACTCAT GGTATGGCA GCACTGCATA ATTCTCTTAC 6622
TGTCATGCCA TCCGTAAGAT GCTTTTCTGT GACTGGTGAG TACTCAACCA AGTCATTCTG 6682
AGAATAGTGT ATGCGGCGAC CGAGTTGCTC TTGCCCGGCG TCAATACGGG ATAATACCGC 6742
GCCACATAGC AGAACTTTAA AAGTGCTCAT CATTGGAAAA CGTTCTTCGG GGCGAAAACT 6802
CTCAAGGATC TTACCGCTGT TGAGATCCAG TTCGATGTAA CCCACTCGTG CACCCAACTG 6862
ATCTTCAGCA TCTTTTACTT TCACCAGCGT TTCTGGGTGA GCAAAAACAG GAAGGCAAAA 6922
TGCCGCAAAA AAGGGAATAA GGGCGACACG GAAATGTTGA ATACTCATAC TCTTCCTTTT 6982
TCAATATTAT TGAAGCATTT ATCAGGGTTA TTGTETCATG AGCGGATACA TATTTGAATG 7042
TATTTAGAAA AATAAACAAA TAGGGGTTC GCGCACATTT CCCCAGAAAAG TGCCACCTGA 7102
CGTC 7106

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 367 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

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Met Leu Pro Gly Leu Ala Leu Leu Leu Leu Ala Ala Trp Thr Ala Arg
 1             5             10             15

Ala Leu Glu Val Pro Thr Asp Gly Asn Ala Gly Leu Leu Ala Glu Pro
      20             25             30

Gln Ile Ala Met Phe Cys Gly Arg Leu Asn Met His Met Asn Val Gln
      35             40             45

Asn Gly Lys Trp Asp Ser Asp Pro Ser Gly Thr Lys Thr Cys Ile Asp
      50             55             60

Thr Lys Glu Thr His Val Thr Gly Gly Ser Ala Gly His Thr Thr Ala
      65             70             75             80

Gly Leu Val Arg Leu Leu Ser Pro Gly Ala Lys Gln Asn Ile Gln Leu
      85             90             95

Ile Asn Thr Asn Gly Ser Trp His Ile Asn Ser Thr Ala Leu Asn Cys
      100            105            110

Asn Glu Ser Leu Asn Thr Gly Trp Leu Ala Gly Leu Phe Tyr His His
      115            120            125

Lys Phe Asn Ser Ser Gly Cys Pro Glu Arg Leu Ala Ser Cys Arg Arg
      130            135            140

Leu Thr Asp Phe Ala Gln Gly Gly Gly Pro Ile Ser Tyr Ala Asn Gly
      145            150            155            160

Ser Gly Leu Asp Glu Arg Pro Tyr Cys Trp His Tyr Pro Pro Arg Pro
      165            170            175

Cys Gly Ile Val Pro Ala Lys Ser Val Cys Gly Pro Val Tyr Cys Phe
      180            185            190

Thr Pro Ser Pro Val Val Val Gly Thr Thr Asp Arg Ser Gly Ala Pro
      195            200            205

Thr Tyr Ser Trp Gly Ala Asn Asp Thr Asp Val Phe Val Leu Asn Asn
      210            215            220

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65

Thr Arg Pro Pro Leu Gly Asn Trp Phe Gly Cys Thr Trp Met Asn Ser
225 230 235 240

Thr Gly Phe Thr Lys Val Cys Gly Ala Pro Pro Cys Val Ile Gly Gly
245 250 255

Val Gly Asn Asn Thr Leu Leu Cys Pro Thr Asp Cys Phe Arg Lys His
260 265 270

Pro Glu Ala Thr Tyr Ser Arg Cys Gly Ser Gly Pro Trp Ile Thr Pro
275 280 285

Arg Cys Met Val Asp Tyr Pro Tyr Arg Leu Trp His Tyr Pro Cys Thr
290 295 300

Ile Asn Tyr Thr Ile Phe Lys Val Arg Met Tyr Val Gly Gly Val Glu
305 310 315 320

His Arg Leu Glu Ala Ala Cys Asn Trp Thr Arg Gly Glu Arg Cys Asp
325 330 335

Leu Glu Asp Arg Asp Arg Ser Glu Leu Ser Pro Leu Leu Leu Ser Thr
340 345 350

Thr Gln Trp Gln Val Leu Pro Cys Ser Phe Thr Thr Leu Pro Ala
355 360 365

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 4810 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: circular

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 2227..2910

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

GCCTAATCTG CTGCTTGCAA ACAAAAAAAC CACCGCTACC AGCGGTGGTT TGTTTGCCGG	60
ATCAAGAGCT ACCAACTCTT TTTCCGAAGG TAACTGGCTT CAGCAGAGCG CAGATACCAA	120
ATACTGTCCT TCTAGTGTAG CCGTAGTTAG GCCACCACTT CAAGAACTCT GTAGCACCGC	180
CTACATACCT CGCTCTGCTA ATCCTGTTAC CAGTGGCTGC TGCCAGTGGC GATAAGTCGT	240
GTCTTACCGG GTTGGACTCA AGACGATAGT TACCGGATAA GGCGCAGCGG TCGGGCTGAA	300

CGGGGGGTTTC GTGCACACAG CCCAGCTTGG AGCGAACGAC CTACACCGAA CTGAGATACC	360
TACAGCGTGA GCATTGAGAA AGCGCCACGC TTCCCGAAGG GAGAAAGGCG GACAGGTATC	420
CGGTAAGCGG CAGGGTCGGA ACAGGAGAGC GCACGAGGGA GCTTCCAGGG GGAAACGCCT	480
GGTATCTTTA TAGTCCTGTC GGGTTTCGCC ACCTCTGACT TGAGCGTCGA TTTTGTGAT	540
GCTCGTCAGG GGGGCGGAGC CTATGGAAAA ACGCCAGCAA CGCAAGCTAG CTTCTAGCTA	600
GAAATTGTAA ACGTTAATAT TTTGTTAAAA TTCGCGTTAA ATTTTTGTGA AATCAGCTCA	660
TTTTTTAACC AATAGGCCGA AATCGGCAAA ATCCCTTATA AATCAAAAGA ATAGCCCGAG	720
ATAGGGTTGA GTGTTGTTCC AGTTTGGAAAC AAGAGTCCAC TATTAAAGAA CGTGGAAGTCC	780
AACGTCAAAG GGCGAAAAAC CGTCTATCAG GGCGATGGCC GCCCACTACG TGAACCATCA	840
CCCAAATCAA GTTTTTTGGG GTCGAGGTGC CGTAAAGCAC TAAATCGGAA CCCTAAAGGG	900
AGCCCCGAT TTAGAGCTTG ACGGGGAAAG CCGGCGAACG TGGCGAGAAA GGAAGGGAAG	960
AAAGCGAAAG GAGCGGGCGC TAGGGCGCTG GCAAGTGTAG CGGTCACGCT GCGCGTAACC	1020
ACCACACCCG CCGCGCTTAA TCGCCGCTA CAGGGCGCGT ACTATGGTTG CTTTGACGAG	1080
ACCGTATAAC GTGCTTTCCT CGTTGGAATC AGAGCGGGAG CTAAACAGGA GGCCGATTAA	1140
AGGGATTTTA GACAGGAACG GTACGCCAGC TGGATCACCG CGGTCTTTCT CAACGTAACA	1200
CTTTACAGCG GCGCGTCATT TGATATGATG CGCCCCGCTT CCCGATAAGG GAGCAGGCCA	1260
GTAAAAGCAT TACCCGTGGT GGGGTTCCTG AGCGGCCAAA GGGAGCAGAC TCTAAATCTG	1320
CCGTCATCGA CTTCGAAGGT TCGAATCCTT CCCCCACCAC CATCACTTTC AAAAGTCCGA	1380
AAGAATCTGC TCCCTGCTTG TGTGTTGGAG GTCGCTGAGT AGTGCGCGAG TAAAATTTAA	1440
GCTACAACAA GGCAAGGCTT GACCGACAAT TGCATGAAGA ATCTGCTTAG GGTAGGCGT	1500
TTTGCGCTGC TTCGCGATGT ACGGGCCAGA TATACGCGTT GACATTGATT ATTGACTAGT	1560
TATTAATAGT AATCAATTAC GGGGTCATTA GTTCATAGCC CATATATGGA GTTCCGCGTT	1620
ACATAACTTA CGGTAAATGG CCCGCCTGGC TGACCGCCCA ACGACCCCCG CCCATTGACG	1680
TCAATAATGA CGTATGTTCC CATAGTAACG CCAATAGGGA CTTTCCATTG ACGTCAATGG	1740
GTGGACTATT TACGGTAAAC TGCCCACTTG GCAGTACATC AAGTGTATCA TATGCCAAGT	1800
ACGCCCCCTA TTGACGTCAA TGACGGTAAA TGGCCCGCCT GGCATTATGC CCAGTACATG	1860
ACCTTATGGG ACTTTCCTAC TTGGCAGTAC ATCTACGTAT TAGTCATCGC TATTACCATG	1920
GTGATGCGGT TTTGGCAGTA CATCAATGGG CGTGGATAGC GGTTTGACTC ACGGGGATTT	1980

CCAAGTCTCC ACCCCATTGA CGTCAATGGG AGTTTGT TTTT GGCACCAAAA TCAACGGGAC	2040
TTTCCAAAAT GTCGTAACAA CTCCGCCCCA TTGACGCAAA TGGGCGGTAG GCGTGTACGG	2100
TGGGAGGTCT ATATAAGCAG AGCTCTCTGG CTAAGTAGAG AACCCACTGC TTAAGTGGCT	2160
TATCGAAATT AATACGACTC ACTATAGGGA GACCGGAAGC TTGGTACCGA GCTCGGATCT	2220
GCCACC ATG GCA ACA GGA TCA AGA ACA TCA CTG CTG CTG GCA TTT GGA	2268
Met Ala Thr Gly Ser Arg Thr Ser Leu Leu Leu Ala Phe Gly	
1 5 10	
CTG CTG TGT CTG CCA TGG CTG CAA GAA GGA TCA GCA GCA GCA GCA GCG	2316
Leu Leu Cys Leu Pro Trp Leu Gln Glu Gly Ser Ala Ala Ala Ala Ala	
15 20 25 30	
AAT TCG GAT CCC TAC CAA GTG CGC AAT TCC TCG GGG CTT TAC CAT GTC	2364
Asn Ser Asp Pro Tyr Gln Val Arg Asn Ser Ser Gly Leu Tyr His Val	
35 40 45	
ACC AAT GAT TGC CCT AAT TCG AGT ATT GTG TAC GAG GCG GCC GAT GCC	2412
Thr Asn Asp Cys Pro Asn Ser Ser Ile Val Tyr Glu Ala Ala Asp Ala	
50 55 60	
ATC CTA CAC ACT CCG GGG TGT GTC CCT TGC GTT CGC GAG GGT AAC GCC	2460
Ile Leu His Thr Pro Gly Cys Val Pro Cys Val Arg Glu Gly Asn Ala	
65 70 75	
TCG AGG TGT TGG GTG GCG GTG ACC CCC ACG GTG GCC ACC AGG GAC GGC	2508
Ser Arg Cys Trp Val Ala Val Thr Pro Thr Val Ala Thr Arg Asp Gly	
80 85 90	
AAA CTC CCC ACA ACG CAG CTT CGA CGT CAT ATC GAT CTG CTC GTC GGG	2556
Lys Leu Pro Thr Thr Gln Leu Arg Arg His Ile Asp Leu Leu Val Gly	
95 100 105 110	
AGC GCC ACC CTC TGC TCG GCC CTC TAC GTG GGG GAC CTG TGC GGG TCT	2604
Ser Ala Thr Leu Cys Ser Ala Leu Tyr Val Gly Asp Leu Cys Gly Ser	
115 120 125	
GTC TTT CTT GTT GGT CAA CTG TTT ACC TTC TCT CCC AGG CGC CAC TGG	2652
Val Phe Leu Val Gly Gln Leu Phe Thr Phe Ser Pro Arg Arg His Trp	
130 135 140	
ACG ACG CAA GAC TGC AAT TGT TCT ATC TAT CCC GGT CAT ATA ACG GGT	2700
Thr Thr Gln Asp Cys Asn Cys Ser Ile Tyr Pro G. His Ile Thr Gly	
145 150 155	
CAT CGT ATG GCA TGG GAT ATG ATG ATG AAC TGG TCC CCT ACG GCA GCG	2748
His Arg Met Ala Trp Asp Met Met Met Asn Trp Ser Pro Thr Ala Ala	
160 165 170	
TTG GTG GTA GCT CAG CTG CTC CGG ATC CCA CAA GCC ATC TTG GAC ATG	2796
Leu Val Val Ala Gln Leu Leu Arg Ile Pro Gln Ala Ile Leu Asp Met	

175	180	185	190	
ATC GCT GGT GCC CAC TGG GGA GTC CTG GCG GGC ATA GCG TAT TTC TCC				2844
Ile Ala Gly Ala His Trp Gly Val Leu Ala Gly Ile Ala Tyr Phe Ser				
195	200	205		
ATG GTG GGG AAC TGG GCG AAG GTC CTG GTA GTG CTG CTG CTA TTT GCC				2892
Met Val Gly Asn Trp Ala Lys Val Leu Val Val Leu Leu Leu Phe Ala				
210	215	220		
GGC GTT GAC GCG GAG ATC TAATCTAGAG GGCCCTATTC TATAGTGTCA				2940
Gly Val Asp Ala Glu Ile				
225				
CCTAAATGCT AGAGGATCTT TGTGAAGGAA CCTTACTTCT GTGGTGTGAC ATAATTGGAC				3000
AAACTACCTA CAGAGATTTA AAGCTCTAAG GTAAATATAA AATTTTAAAG TGTATAATGT				3060
GTTAAACTAC TGATTCTAAT TGTTTGTGTA TTTTAGATTTC CAACCTATGG AACTGATGAA				3120
TGGGAGCAGT GGTGGAATGC CTTTAATGAG GAAAACCTGT TTTGCTCAGA AGAAATGCCA				3180
TCTAGTGATG ATGAGGCTAC TGCTGACTCT CAACATTCTA CTCCTCCAAA AAAGAAGAGA				3240
AAGGTAGAAG ACCCCAAGGA CTTTCCTTCA GAATTGCTAA GTTTTTTGAG TCATGCTGTG				3300
TTTAGTAATA GAACTCTTGC TTGCTTTGCT ATTTACACCA CAAAGGAAAA AGCTGCACTG				3360
CTATACAAGA AAATTATGGA AAAATATTCT GTAACCTTTA TAAGTAGGCA TAACAGTTAT				3420
AATCATAACA TACTGTTTTT TCTTACTCCA CACAGGCATA GAGTGTCTGC TATTAATAAC				3480
TATGCTCAAA AATTGTGTAC CTTTAGCTTT TTAATTTGTA AAGGGGTAA TAAGGAATAT				3540
TTGATGTATA GTGCCTTGAC TAGAGATCAT AATCAGCCAT ACCACATTTG TAGAGGTTTT				3600
ACTTGCTTTA AAAAACCTCC CACACCTCCC CCTGAACCTG AAACATAAAA TGAATGCAAT				3660
TGTTGTTGTT AACTTGTTA TTGCAGCTTA TAATGGTTAC AAATAAAGCA ATAGCATCAC				3720
AAATTTTACA AATAAAGCAT TTTTTTCACT GCATTCTAGT TGTGGTTTGT CCAAATCAT				3780
CAATGTATCT TATCATGTCT GGATCGATCC CGCCATGGTA TCAACGCCAT ATTTCTATTT				3840
ACAGTAGGGA CCTCTTCGTT GTGTAGGTAC CGCTGTATTC CTAGGGAAAT AGTAGAGGCA				3900
CCTTGAAGT TCTGCATCAG CCATATAGCC CCCGCTGTTT GACTTACAAA CACAGGCACA				3960
GTACTGACAA ACCCATACAC CTCCTCTGAA ATACCCATAG TTGCTAGGGC TGTCTCCGAA				4020
CTCATTACAC CCTCCAAAGT CAGAGCTGTA ATTTGCGCAT CAAGGGCAGC GAGGGCTTCT				4080
CCAGATAAAA TAGCTTCTGC CGAGAGTCCC GTAAGGGTAG AACTTCAGC TAATCCCTCG				4140
ATGAGGTCTA CTAGAATAGT CAGTGCGGCT CCCATTTTGA AAATTCACCT ACTTGATCAG				4200

CTTCAGAAGA TGGCGGAGGG CCTCCAACAC AGTAATTTTC CTCCCGACTC TTAAAATAGA 4260
 AAATGTCAAG TCAGTTAAGC AGGAAGTGA CTAAGTACG CAGCTGGCCG TGCACATCC 4320
 TCTTTTAATT AGTTGCTAGG CAACGCCCTC CAGAGGGCGT GTGGTTTTGC AAGAGGAAGC 4380
 AAAAGCCTCT CCACCCAGGC CTAGAATGTT TCCACCCAAT CATTACTATG ACAACAGCTG 4440
 TTTTTTTTAG TATTAAGCAG AGGCCGGGGA CCCCTGGCCC GCTTACTCTG GAGAAAAAGA 4500
 AGAGAGGCAT TGTAGAGGCT TCCAGAGGCA ACTTGTCAAA ACAGGACTGC TTCTATTTCT 4560
 GTCACACTGT CTGGCCCTGT CACAAGGTCC AGCACCTCCA TACCCCTTT AATAAGCAGT 4620
 TTGGGAACGG GTGCGGGTCT TACTCCGCC ATCCCGCCCC TAACTCCGCC CAGTTCCGCC 4680
 CATCTCCGC CCCATGGCTG ACTAATTTTT TTTATTTATG CAGAGGCCGA GGCCGCCTCG 4740
 GCCTCTGAGC TATTCAGAA GTAGTGAGGA GGCTTTTTTG GAGGCCTAGG CTTTTGCAAA 4800
 AAGCTAATTC 4810

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 228 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Met Ala Thr Gly Ser Arg Thr Ser Leu Leu Leu Ala Phe Gly Leu Leu
 1 5 10 15
 Cys Leu Pro Trp Leu Gln Glu Gly Ser Ala Ala Ala Ala Asn Ser
 20 25 30
 Asp Pro Tyr Gln Val Arg Asn Ser Ser Gly Leu Tyr His Val Thr Asn
 35 40 45
 Asp Cys Pro Asn Ser Ser Ile Val Tyr Glu Ala Ala Asp Ala Ile Leu
 50 55 60
 His Thr Pro Gly Cys Val Pro Cys Val Arg Glu Gly Asn Ala Ser Arg
 65 70 75 80
 Cys Trp Val Ala Val Thr Pro Thr Val Ala Thr Arg Asp Gly Lys Leu
 85 90 95
 Pro Thr Thr Gln Leu Arg Arg His Ile Asp Leu Leu Val Gly Ser Ala
 100 105 110

Thr Leu Cys Ser Ala Leu Tyr Val Gly Asp Leu Cys Gly Ser Val Phe
 115 120 125
 Leu Val Gly Gln Leu Phe Thr Phe Ser Pro Arg Arg His Trp Thr Thr
 130 135 140
 Gln Asp Cys Asn Cys Ser Ile Tyr Pro Gly His Ile Thr Gly His Arg
 145 150 155 160
 Met Ala Trp Asp Met Met Met Asn Trp Ser Pro Thr Ala Ala Leu Val
 165 170 175
 Val Ala Gln Leu Leu Arg Ile Pro Gln Ala Ile Leu Asp Met Ile Ala
 180 185 190
 Gly Ala His Trp Gly Val Leu Ala Gly Ile Ala Tyr Phe Ser Met Val
 195 200 205
 Gly Asn Trp Ala Lys Val Leu Val Val Leu Leu Leu Phe Ala Gly Val
 210 215 220
 Asp Ala Glu Ile
 225

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 5323 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: circular

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 2227..3423

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

GCGTAATCTG CTGCTTGCAA ACAAAAAAAC CACCGCTACC AGCGGTGGTT TGTTCGCCGG 60
 ATCAAGAGCT ACCAACTCTT TTTCCGAAGG TAACTGGCTT CAGCAGAGCG CAGATACCAA 120
 ATACTGTCCT TCTAGTGTAG CCGTAGTTAG GCCACCACTT CAAGAACTCT GTAGCACCGC 180
 CTACATACCT CGCTCTGCTA ATCCTGTTAC CAGTGGCTGC TGCCAGTGGC GATAAGTCGT 240
 GTCTTACCGG GTTGGACTCA AGACGATAGT TACCGGATAA GGCGCAGCGG TCGGGCTGAA 300
 CGGGGGGTTT GTGCACACAG CCCAGCTTGG AGCGAACGAC CTACACCGAA CTGAGATACC 360

TACAGCGTGA GCATTGAGAA AGCGCCACGC TTCCCGAAGG GAGAAAGGCG GACAGGTATC	420
CGGTAAGCGG CAGGGTCGGA ACAGGAGAGC GCACGAGGGA GCTTCCAGGG GGAAACGCCT	480
GGTATCTTTA TAGTCCTGTC GGGTTTCGCC ACCTCTGACT TGAGCGTCGA TTTTGTGAT	540
GCTCGTCAGG GGGGCGGAGC CTATGGAAAA ACGCCAGCAA CGCAAGCTAG CTTCTAGCTA	600
GAAATTGTAA ACGTTAATAT TTTGTAAAA TTCGCGTTAA ATTTTGTTA AATCAGCTCA	660
TTTTTTAACC AATAGGCCGA AATCGGCAA ATCCCTTATA AATCAAAAGA ATAGCCCGAG	720
ATAGGGTTGA GTGTTGTTCC AGTTTGGAAC AAGAGTCCAC TATTAAAGAA CGTGGACTCC	780
AACGTCAAAG GCGGAAAAAC CGTCTATCAG GGCGATGGCC GCCCACTACG TGAACCATCA	840
CCCAAATCAA GTTTTTTGGG GTCGAGGTGC CGTAAAGCAC TAAATCGGAA CCTTAAAGGG	900
AGCCCCCGAT TTAGAGCTTG ACGGGGAAAG CCGGCGAACG TGGCGAGAAA GGAAGGGAAG	960
AAAGCGAAAG GAGCGGGCGC TAGGGCGCTG GCAAGTGTAG CGGTCACGCT GCGCGTAACC	1020
ACCACACCCG CCGCGCTTAA TGCGCCGCTA CAGGGCGCGT ACTATGGTTG CTTTGACGAG	1080
ACCGTATAAC GTGCTTTCCT CGTTGGAATC AGAGCGGGAG CTAAACAGGA GGCCGATTAA	1140
AGGGATTTTA GACAGGAACG GTACGCCAGC TGGATCACCG CGGTCTTTCT CAACGTAACA	1200
CTTTACAGCG GCGCGTCATT TGATATGATG CGCCCCGCTT CCCGATAAGG GAGCAGGCCA	1260
GTAAAAGCAT TACCCGTGGT GGGGTTCCCG AGCGGCCAAA GGGAGCAGAC TCTAAATCTG	1320
CCGTCATCGA CTTGGAAGGT TCGAATCCTT CCCCCACCAC CATCACTTTC AAAAGTCCGA	1380
AAGAATCTGC TCCCTGCTTG TGTGTTGGAG GTCGCTGAGT AGTGCGCGAG TAAAATTTAA	1440
GCTACAACAA GGCAAGGCTT GACCGACAAT TGCATGAAGA ATCTGCTTAG GGTTAGGCGT	1500
TTTGCGCTGC TTCGCGATGT ACGGGCCAGA TATACGCGTT GACATTGATT ATTGACTAGT	1560
TATTAATAGT AATCAATTAC GGGGTCATTA GTTCATAGCC CATATATGGA GTTCCGCGTT	1620
ACATAACTTA CGGTAAATGG CCCGCTGGC TGACCGCCCA ACGACCCCCG CCCATTGACG	1680
TCAATAATGA CGTATGTTCC CATAGTAACG CCAATAGGGA CTTTCCATTG ACGTCAATGG	1740
GTGGACTATT TACGGTAAAC TGCCCACTTG GCAGTACATC AAGTGTATCA TATGCCAAGT	1800
ACGCCCCCTA TTGACGTCAA TGACGGTAAA TGGCCCGCCT GGCATTATGC CCAGTACATG	1860
ACCTTATGGG ACTTTCCTAC TTGGCAGTAC ATCTACGTAT TAGTCATCGC TATTACCATG	1920
GTGATGCGGT TTTGGCAGTA CATCAATGGG CGTGGATAGC GGTTTGACTC ACGGGGATTT	1980
CCAAGTCTCC ACCCCATTGA CGTCAATGGG AGTTTGTTTT GGCACCAAAA TCAACGGGAC	2040

TTTCCAAAAT GTCGTAACAA CTCCGCCCCA TTGACGCAAA TGGGCGGTAG GCGTGTACGG 2100
 TGGGAGGTCT ATATAGCAG AGCTCTCTG3 CTAAGTAGAG AACCCACTGC TTAAGTGGCT 2160
 TATCGAAATT AATACGACTC ACTATAGGGA GACCGGAAGC TTGGTACCGA GCTCGGATCT 2220
 GCCACC ATG GCA ACA GGA TCA AGA ACA TCA CTG CTG CTG GCA TTT GGA 2268
 Met Ala Thr Gly Ser Arg Thr Ser Leu Leu Ala Phe Gly
 1 5 10
 CTG CTG TGT CTG CCA TGG CTG CAA GAA GGA TCA GCA GCA GCA GCA GCG 2316
 Leu Leu Cys Leu Pro Trp Leu Gln Glu Gly Ser Ala Ala Ala Ala Ala
 15 20 25 30
 AAT TCA GAA ACC CAC GTC ACC GGG GGA AGT GCC GGC CAC ACC ACG GCT 2364
 Asn Ser Glu Thr His Val Thr Gly Gly Ser Ala Gly His Thr Thr Ala
 35 40 45
 GGG CTT GTT CGT CTC CTT TCA CCA GGC GCC AAG CAG AAC ATC CAA CTG 2412
 Gly Leu Val Arg Leu Leu Ser Pro Gly Ala Lys Gln Asn Ile Gln Leu
 50 55 60
 ATC AAC ACC AAC GGC AGT TGG CAC ATC AAT AGC ACG GCC TTG AAC TGC 2460
 Ile Asn Thr Asn Gly Ser Trp His Ile Asn Ser Thr Ala Leu Asn Cys
 65 70 75
 AAT GAA AGC CTT AAC ACC GGC TGG TTA GCA GGC CTC TTC TAT CAC CAC 2508
 Asn Glu Ser Leu Asn Thr Gly Trp Leu Ala Gly Leu Phe Tyr His His
 80 85 90
 AAA TTC AAC TCT TCA GGT TGT CCT GAG AGG TTG GCC AGC TGC CGA CGC 2556
 Lys Phe Asn Ser Ser Gly Cys Pro Glu Arg Leu Ala Ser Cys Arg Arg
 95 100 105 110
 CTT ACC GAT TTT GCC CAG GGC GGG GGT CCT ATC AGT TAC GCC AAC GGA 2604
 Leu Thr Asp Phe Ala Gln Gly Gly Gly Pro Ile Ser Tyr Ala Asn Gly
 115 120 125
 AGC GGC CTC GAT GAA CGC CCC TAC TGC TGG CAC TAC CCT CCA AGA CCT 2652
 Ser Gly Leu Asp Glu Arg Pro Tyr Cys Trp His Tyr Pro Pro Arg Pro
 130 135 140
 TGT GGC ATT GTG CCC GCA AAG AGC GTG TGT GGC CCG GTA TAT TGC TTC 2700
 Cys Gly Ile Val Pro Ala Lys Ser Val Cys Gly Pro Val Tyr Cys Phe
 145 150 155
 ACT CCC AGC CCC GTG GTG GTG GGA ACG ACC GAC AGG TCG GGC GCG CCT 2748
 Thr Pro Ser Pro Val Val Val Gly Thr Thr Asp Arg Ser Gly Ala Pro
 160 165 170
 ACC TAC AGC TGG GGT GCA AAT GAT ACG GAT GTC TTT GTC CTT AAC AAC 2796
 Thr Tyr Ser Trp Gly Ala Asn Asp Thr Asp Val Phe Val Leu Asn Asn
 175 180 185 190

73

ACC AGG CCA CCG CTG GGC AAT TGG TTC GGT TGC ACC TGG ATG AAC TCA	2844
Thr Arg Pro Pro Leu Gly Asn Trp Phe Gly Cys Thr Trp Met Asn Ser	
195 200 205	
ACT GGA TTC ACC AAA GTG TGC GGA GCG CCC CCT TGT GTC ATC GGA GGG	2892
Thr Gly Phe Thr Lys Val Cys Gly Ala Pro Pro Cys Val Ile Gly Gly	
210 215 220	
GTG GGC AAC AAC ACC TTG CTC TGC CCC ACT GAT TGC TTC CGC AAG CAT	2940
Val Gly Asn Asn Thr Leu Leu Cys Pro Thr Asp Cys Phe Arg Lys His	
225 230 235	
CCG GAA GCC ACA TAC TCT CGG TGC GGC TCC GGT CCC TGG ATT ACA CCC	2988
Pro Glu Ala Thr Tyr Ser Arg Cys Gly Ser Gly Pro Trp Ile Thr Pro	
240 245 250	
AGG TGC ATG GTC GAC TAC CCG TAT AGG CTT TGG CAC TAT CCT TGT ACC	3036
Arg Cys Met Val Asp Tyr Pro Tyr Arg Leu Trp His Tyr Pro Cys Thr	
255 260 265 270	
ATC AAT TAC ACC ATA TTC AAA GTC AGG ATG TAC GTG GGA GGG GTC GAG	3084
Ile Asn Tyr Thr Ile Phe Lys Val Arg Met Tyr Val Gly Gly Val Glu	
275 280 285	
CAC AGG CTG GAA GCG GCC TGC AAC TGG ACG CGG GGC GAA CGC TGT GAT	3132
His Arg Leu Glu Ala Ala Cys Asn Trp Thr Arg Gly Glu Arg Cys Asp	
290 295 300	
CTG GAA GAC AGG GAC AGG TCC GAG CTC AGC CCG TTA CTG CTG TCC ACC	3180
Leu Glu Asp Arg Asp Arg Ser Glu Leu Ser Pro Leu Leu Ser Thr	
305 310 315	
ACG CAG TGG CAG GTC CTT CCG TGT TCT TTC ACG ACC CTG CCA GCC TTG	3228
Thr Gln Trp Gln Val Leu Pro Cys Ser Phe Thr Thr Leu Pro Ala Leu	
320 325 330	
TCC ACC GGC CTC ATC CAC CTC CAC CAG AAC ATT GTG GAC GTG CAG TAC	3276
Ser Thr Gly Leu Ile His Leu His Gln Asn Ile Val Asp Val Gln Tyr	
335 340 345 350	
TTG TAC GGG GTA GGG TCA AGC ATC GCG TCC TGG GCT ATT AAG TGG GAG	3324
Leu Tyr Gly Val Gly Ser Ser Ile Ala Ser Trp Ala Ile Lys Trp Glu	
355 360 365	
TAC GAC GTT CTC CTG TTC CTT CTG CTT GCA GAC GCG CGC GTT TGC TCC	3372
Tyr Asp Val Leu Leu Phe Leu Leu Leu Ala Asp Ala Arg Val Cys Ser	
370 375 380	
TGC TTG TGG ATG ATG TTA CTC ATA TCC CAA GCG GAG GCG GCT TTG GAG	3420
Cys Leu Trp Met Met Leu Leu Ile Ser Gln Ala Glu Ala Ala Leu Glu	
385 390 395	
AAC TAATCTAGAG GGCCCTATTC TATAGTGTCA CCTAAATGCT AGAGGATCTT	3473
Asn	

TGTGAAGGAA CCTTACTTCT GTGGTGTGAC ATAATTGGAC AAACCTACCTA CAGAGATTTA	3533
AAGCTCTAAG GTAAATATAA AATTTTTTAAG TGTATAATGT GTTAAACTAC TGATTCTAAT	3593
TGTTTGTGTA TTTTAGATTTC CAACCTATGG AACTGATGAA TGGGAGCAGT GGTGGAATGC	3653
CTTTAATGAG GAAAACCTGT TTTGCTCAGA AGAAATGCCA TCTAGTGATG ATGAGGCTAC	3713
TGCTGACTCT CAACATTCTA CTCCTCCAAA AAAGAAGAGA AAGGTAGAAG ACCCCAAGGA	3773
CTTTCCTTCA GAATTGCTAA GTTTTTTGAG TCATGCTGTG TTTAGTAATA GAACTCTTGC	3833
TTGCTTTGCT ATTTACACCA CAAAGGAAAA AGCTGCACTG CTATACAAGA AAATTATGGA	3893
AAAATATTCT GTAACCTTTA TAAGTAGGCA TAACAGTTAT AATCATAACA TACTGTTTTT	3953
TCTTACTCCA CACAGGCATA GAGTGTCTGC TATTAATAAC TATGCTCAAA AATTGTGTAC	4013
CTTTAGCTTT TTAATTTGTA AAGGGGTTAA TAAGGAATAT TTGATGTATA GTGCCTTGAC	4073
TAGAGATCAT AATCAGCCAT ACCACATTG TAGAGGTTTT ACTTGCTTTA AAAAACCTCC	4133
CACACCTCCC CCTGAACCTG AAACATAAAA TGAATGCAAT TGTGTGTGTT AACTTGTTTA	4193
TTGCAGCTTA TAATGGTTAC AAATAAAGCA ATAGCATCAC AAATTTTACA AATAAAGCAT	4253
TTTTTTCACCT GCATTCTAGT TGTGGTTTTGT CCAAACTCAT CAATGTATCT TATCATGTCT	4313
GGATCGATCC CGCCATGGTA TCAACGCCAT ATTTCTATTT ACAGTAGGGA CCTCTTCGTT	4373
GTGTAGGTAC CGCTGTATTC CTAGGGAAAT AGTAGAGGCA CTTTGAACCTG TCTGCATCAG	4433
CCATATAGCC CCCGCTGTTC GACTTACAAA CACAGGCACA GTACTGACAA ACCCATAACAC	4493
CTCCTCTGAA ATACCCATAG TTGCTAGGGC TGTCTCCGAA CTCATTACAC CCTCCAAAGT	4553
CAGAGCTGTA ATTTTCGCCAT CAAGGGCAGC GAGGGCTTCT CCAGATAAAA TAGCTTCTGC	4613
CGAGAGTCCC GTAAGGGTAG ACACTTCAGC TAATCCCTCG ATGAGGTCTA CTAGAATAGT	4673
CAGTGCGGCT CCCATTTTGA AAATTCACCTT ACTTGATCAG CTTTCAAGA TGGCGGAGGG	4733
CCTCCAACAC AGTAATTTTC CTCCCGACTC TTAAAATAGA AAATGTCAAG TCAGTTAAGC	4793
AGGAAGTGGA CTAACCTGACG CAGCTGGCCG TGCACATCC TCTTTTAATT AGTTGCTAGG	4853
CAACGCCCTC CAGAGGGCGT GTGGTTTTGC AAGAGGAAGC AAAAGCCTCT CCACCCAGGC	4913
CTAGAATGTT TCCACCCAAT CATTACTATG ACAACAGCTG TTTTTTTTAG TATTAAGCAG	4973
AGGCCGGGGA CCCCTGGCCC GCTTACTCTG GAGAAAAAGA AGAGAGGCAT TGTAGAGGCT	5033
TCCAGAGGCA ACTTGTCAAA ACAGGACTGC TTCTATTTCT GTCACACTGT CTGGCCCTGT	5093

75

CACAAGGTCC AGCACCTCCA TACCCCTTTT AATAAGCAGT TTGGGAACGG GTGCGGGTCT 5153
 TACTCCGCCC ATCCCGCCCC TAACTCCGCC CAGTTCCGCC CATTCTCCGC CCCATGGCTG 5213
 ACTAATTTTT TTTATTATG CAGAGGCCGA GCGCGCCTCG GCCTCTGAGC TATTCCAGAA 5273
 GTAGTGAGGA GGCTTTTTTG GAGGCCTAGG CTTTGTCAAA AAGCTAATTC 5323

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 399 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Met Ala Thr Gly Ser Arg Thr Ser Leu Leu Leu Ala Phe Gly Leu Leu
 1 5 10 15
 Cys Leu Pro Trp Leu Gln Glu Gly Ser Ala Ala Ala Ala Ala Asn Ser
 20 25 30
 Glu Thr His Val Thr Gly Gly Ser Ala Gly His Thr Thr Ala Gly Leu
 35 40 45
 Val Arg Leu Leu Ser Pro Gly Ala Lys Gln Asn Ile Gln Leu Ile Asn
 50 55 60
 Thr Asn Gly Ser Trp His Ile Asn Ser Thr Ala Leu Asn Cys Asn Glu
 65 70 75 80
 Ser Leu Asn Thr Gly Trp Leu Ala Gly Leu Phe Tyr His His Lys Phe
 85 90 95
 Asn Ser Ser Gly Cys Pro Glu Arg Leu Ala Ser Cys Arg Arg Leu Thr
 100 105 110
 Asp Phe Ala Gln Gly Gly Gly Pro Ile Ser Tyr Ala Asn Gly Ser Gly
 115 120 125
 Leu Asp Glu Arg Pro Tyr Cys Trp His Tyr Pro Pro Arg Pro Cys Gly
 130 135 140
 Ile Val Pro Ala Lys Ser Val Cys Gly Pro Val Tyr Cys Phe Thr Pro
 145 150 155 160
 Ser Pro Val Val Val Gly Thr Thr Asp Arg Ser Gly Ala Pro Thr Tyr
 165 170 175
 Ser Trp Gly Ala Asn Asp Thr Asp Val Phe Val Leu Asn Asn Thr Arg
 180 185 190

Pro Pro Leu Gly Asn Trp Phe Gly Cys Thr Trp Met Asn Ser Thr Gly
 195 200 205
 Phe Thr Lys Val Cys Gly Ala Pro Pro Cys Val Ile Gly Gly Val Gly
 210 215 220
 Asn Asn Thr Leu Leu Cys Pro Thr Asp Cys Phe Arg Lys His Pro Glu
 225 230 235 240
 Ala Thr Tyr Ser Arg Cys Gly Ser Gly Pro Trp Ile Thr Pro Arg Cys
 245 250 255
 Met Val Asp Tyr Pro Tyr Arg Leu Trp His Tyr Pro Cys Thr Ile Asn
 260 265 270
 Tyr Thr Ile Phe Lys Val Arg Met Tyr Val Gly Gly Val Glu His Arg
 275 280 285
 Leu Glu Ala Ala Cys Asn Trp Thr Arg Gly Glu Arg Cys Asp Leu Glu
 290 295 300
 Asp Arg Asp Arg Ser Glu Leu Ser Pro Leu Leu Leu Ser Thr Thr Gln
 305 310 315 320
 Trp Gln Val Leu Pro Cys Ser Phe Thr Thr Leu Pro Ala Leu Ser Thr
 325 330 335
 Gly Leu Ile His Leu His Gln Asn Ile Val Asp Val Gln Tyr Leu Tyr
 340 345 350
 Gly Val Gly Ser Ser Ile Ala Ser Trp Ala Ile Lys Trp Glu Tyr Asp
 355 360 365
 Val Leu Leu Phe Leu Leu Leu Ala Asp Ala Arg Val Cys Ser Cys Leu
 370 375 380
 Trp Met Met Leu Leu Ile Ser Gln Ala Glu Ala Ala Leu Glu Asn
 385 390 395

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 5125 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: circular

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 2227..3225

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

CGGTAATCTG CTGCTTGCAA ACAAAAAAAC CACCGCTACC AGCGGTGGTT TGTTTGCCGG	60
ATCAAGAGCT ACCAACTCTT TTTCCGAAGG TAACTGGCTT CAGCAGAGCG CAGATACCAA	120
ATACTGTCCT TCTAGTGTAG CCGTAGTTAG GCCACCACTT CAAGAACTCT GTAGCACCGC	180
CTACATACCT CGCTCTGCTA ATCCTGTTAC CAGTGGCTGC TGCCAGTGGC GATAAGTCGT	240
GTCTTACCGG GTTGGA CTCA AGACGATAGT TACCGGATAA GGCGCAGCGG TCGGGCTGAA	300
CGGGGGGTTC GTGCACACAG CCCAGCTTGG AGCGAACGAC CTACACCGAA CTGAGATACC	360
TACAGCGTGA GCATTGAGAA AGCGCCACGC TTCCCGAAGG GAGAAAGGCG GACAGGTATC	420
CGGTAAGCGG CAGGGTCGGA ACAGGAGAGC GCACGAGGGA GCTTCCAGGG GGAAACGCCT	480
GGTATCTTTA TAGTCCTGTC GGGTTTCGCC ACCTCTGACT TGAGCGTCGA TTTTGTGAT	540
GCTCGTCAGG GGGGCGGAGC CTATGGAAAA ACGCCAGCAA CGCAAGCTAG CTTCTAGCTA	600
GAAATTGTAA ACGTTAATAT TTTGTTAAAA TTCGCGTTAA ATTTTGTGTA AATCAGCTCA	660
TTTTTTAACC AATAGGCCGA AATCGGCAAA ATCCCTTATA AATCAAAAGA ATAGCCCGAG	720
ATAGGGTTGA GTGTTGTTCC AGTTTGGAAC AAGAGTCCAC TATTAAAGAA CGTGGACTCC	780
AACGTCAAAG GGCGAAAAAC CGTCTATCAG GCGATGGCC GCCCACTACG TGAACCATCA	840
CCCAAATCAA GTTTTTTGGG GTCGAGGTGC CGTAAAGCAC TAAATCGGAA CCCTAAAGGG	900
AGCCCCCGAT TTAGAGCTTG ACGGGGAAAG CCGGCGAACG TGGCGAGAAA GGAAGGGAAG	960
AAAGCGAAAG GAGCGGGCGC TAGGGCGCTG GCAAGTGTAG CGGTCACGCT GCGCGTAACC	1020
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AGGGATTTTA GACAGGAACG GTACGCCAGC TGGATCACCG CGGTCTTTCT CAACGTAACA	1200
CTTTACAGCG GCGCGTCATT TGATATGATG CGCCCCGCTT CCCGATAAGG GAGCAGGCCA	1260
GTAAAAGCAT TACCCGTGGT GGGGTTCCCG AGCGGCCAAA GGGAGCAGAC TCTAAATCTG	1320
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TTTGCGCTGC TTCGCGATGT ACGGGCCAGA TATACGCGTT GACATTGATT ATTGACTAGT	1560

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 GTGATGCGGT TTTGGCAGTA CATCAATGGG CGTGGATAGC GGTTTGACTC ACGGGGATTT 1980
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 1 5 10
 CTG CTG TGT CTG CCA TGG CTG CAA GAA GGA TCA GCA GCA GCA GCA GCG 2316
 Leu Leu Cys Leu Pro Trp Leu Gln Glu Gly Ser Ala Ala Ala Ala Ala
 15 20 25 30
 AAT TCA GAA ACC CAC GTC ACC GGG GGA AGT GCC GGC CAC ACC ACG GCT 2364
 Asn Ser Glu Thr His Val Thr Gly Gly Ser Ala Gly His Thr Thr Ala
 35 40 45
 GGG CTT GTT CGT CTC CTT TCA CCA GGC GCC AAG CAG AAC ATC CAA CTG 2412
 Gly Leu Val Arg Leu Leu Ser Pro Gly Ala Lys Gln Asn Ile Gln Leu
 50 55 60
 ATC AAC ACC AAC GGC AGT TGG CAC ATC AAT AGC ACG GCC TTG AAC TGC 2460
 Ile Asn Thr Asn Gly Ser Trp His Ile Asn Ser Thr Ala Leu Asn Cys
 65 70 75
 AAT GAA AGC CTT AAC ACC GGC TGG TTA GCA GGG CTC TTC TAT CAC CAC 2508
 Asn Glu Ser Leu Asn Thr Gly Trp Leu Ala Gly Leu Phe Tyr His His
 80 85 90
 AAA TTC AAC TCT TCA GGT TGT CCT GAG AGG TTG GCC AGC TGC CGA CGC 2556
 Lys Phe Asn Ser Ser Gly Cys Pro Glu Arg Leu Ala Ser Cys Arg Arg
 95 100 105 110
 CTT ACC GAT TTT GCC CAG GGC GGG GGT CCT ATC AGT TAC GCC AAC GGA 2604
 Leu Thr Asp Phe Ala Gln Gly Gly Gly Pro Ile Ser Tyr Ala Asn Gly
 115 120 125
 AGC GGC CTC GAT GAA CGC CCC TAC TGC TGG CAC TAC CCT CCA AGA CCT 2652

Ser Gly Leu Asp Glu Arg Pro Tyr Cys Trp His Tyr Pro Pro Arg Pro	
130 135 140	
TGT GGC ATT GTG CCC GCA AAG AGC GTG TGT GGC CCG GTA TAT TGC TTC	2700
Cys Gly Ile Val Pro Ala Lys Ser Val Cys Gly Pro Val Tyr Cys Phe	
145 150 155	
ACT CCC AGC CCC GTG GTG GTG GGA ACG ACC GAC AGG TCG GGC GCG CCT	2748
Thr Pro Ser Pro Val Val Val Gly Thr Thr Asp Arg Ser Gly Ala Pro	
160 165 170	
ACC TAC AGC TGG GGT GCA AAT GAT ACG GAT GTC TTT GTC CTT AAC AAC	2796
Thr Tyr Ser Trp Gly Ala Asn Asp Thr Asp Val Phe Val Leu Asn Asn	
175 180 185 190	
ACC AGG CCA CCG CTG GGC AAT TGG TTC GGT TGC ACC TGG ATG AAC TCA	2844
Thr Arg Pro Pro Leu Gly Asn Trp Phe Gly Cys Thr Trp Met Asn Ser	
195 200 205	
ACT GGA TTC ACC AAA GTG TGC GGA GCG CCC CCT TGT GTC ATC GGA GGG	2892
Thr Gly Phe Thr Lys Val Cys Gly Ala Pro Pro Cys Val Ile Gly Gly	
210 215 220	
GTG GGC AAC AAC ACC TTG CTC TGC CCC ACT GAT TGC TTC CGC AAG CAT	2940
Val Gly Asn Asn Thr Leu Leu Cys Pro Thr Asp Cys Phe Arg Lys His	
225 230 235	
CCG GAA GCC ACA TAC TCT CGG TGC GGC TCC GGT CCC TGG ATT ACA CCC	2988
Pro Glu Ala Thr Tyr Ser Arg Cys Gly Ser Gly Pro Trp Ile Thr Pro	
240 245 250	
AGG TGC ATG GTC GAC TAC CCG TAT AGG CTT TGG CAC TAT CCT TGT ACC	3036
Arg Cys Met Val Asp Tyr Pro Tyr Arg Leu Trp His Tyr Pro Cys Thr	
255 260 265 270	
ATC AAT TAC ACC ATA TTC AAA GTC AGG ATG TAC GTG GGA GGG GTC GAG	3084
Ile Asn Tyr Thr Ile Phe Lys Val Arg Met Tyr Val Gly Gly Val Glu	
275 280 285	
CAC AGG CTG GAA GCG GCC TGC AAC TGG ACG CGG GGC GAA CGC TGT GAT	3132
His Arg Leu Glu Ala Ala Cys Asn Trp Thr Arg Gly Glu Arg Cys Asp	
290 295 300	
CTG GAA GAC AGG GAC AGG TCC GAG CTC AGC CCG TTA CTG CTG TCC ACC	3180
Leu Glu Asp Arg Asp Arg Ser Glu Leu Ser Pro Leu Leu Leu Ser Thr	
305 310 315	
ACG CAG TGG CAG GTC CTT CCG TGT TCT TTC ACG ACC CTG CCA GCC	3225
Thr Gln Trp Gln Val Leu Pro Cys Ser Phe Thr Thr Leu Pro Ala	
320 325 330	
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TTTATTTATG CAGAGGCCGA GGCCGCCTCG GCCTCTGAGC TATTCCAGAA GTAGTGAGGA 5085
 GGCTTTTTTG GAGGCCTAGG CTTTTCAGAA AAGCTAATTC 5125

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 333 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

Met Ala Thr Gly Ser Arg Thr Ser Leu Leu Leu Ala Phe Gly Leu Leu
 1 5 10 15
 Cys Leu Pro Trp Leu Gln Glu Gly Ser Ala Ala Ala Ala Ala Asn Ser
 20 25 30
 Glu Thr His Val Thr Gly Gly Ser Ala Gly His Thr Thr Ala Gly Leu
 35 40 45
 Val Arg Leu Leu Ser Pro Gly Ala Lys Gln Asn Ile Gln Leu Ile Asn
 50 55 60
 Thr Asn Gly Ser Trp His Ile Asn Ser Thr Ala Leu Asn Cys Asn Glu
 65 70 75 80
 Ser Leu Asn Thr Gly Trp Leu Ala Gly Leu Phe Tyr His His Lys Phe
 85 90 95
 Asn Ser Ser Gly Cys Pro Glu Arg Leu Ala Ser Cys Arg Arg Leu Thr
 100 105 110
 Asp Phe Ala Gln Gly Gly Gly Pro Ile Ser Tyr Ala Asn Gly Ser Gly
 115 120 125
 Leu Asp Glu Arg Pro Tyr Cys Trp His Tyr Pro Pro Arg Pro Cys Gly
 130 135 140
 Ile Val Pro Ala Lys Ser Val Cys Gly Pro Val Tyr Cys Phe Thr Pro
 145 150 155 160
 Ser Pro Val Val Val Gly Thr Thr Asp Arg Ser Gly Ala Pro Thr Tyr
 165 170 175
 Ser Trp Gly Ala Asn Asp Thr Asp Val Phe Val Leu Asn Asn Thr Arg
 180 185 190
 Pro Pro Leu Gly Asn Trp Phe Gly Cys Thr Trp Met Asn Ser Thr Gly
 195 200 205

Phe Thr Lys Val Cys Gly Ala Pro Pro Cys Val Ile Gly Gly Val Gly
210 215 220

Asn Asn Thr Leu Leu Cys Pro Thr Asp Cys Phe Arg Lys His Pro Glu
225 230 235 240

Ala Thr Tyr Ser Arg Cys Gly Ser Gly Pro Trp Ile Thr Pro Arg Cys
245 250 255

Met Val Asp Tyr Pro Tyr Arg Leu Trp His Tyr Pro Cys Thr Ile Asn
260 265 270

Tyr Thr Ile Phe Lys Val Arg Met Tyr Val Gly Gly Val Glu His Arg
275 280 285

Leu Glu Ala Ala Cys Asn Trp Thr Arg Gly Glu Arg Cys Asp Leu Glu
290 295 300

Asp Arg Asp Arg Ser Glu Leu Ser Pro Leu Leu Leu Ser Thr Thr Gln
305 310 315 320

Trp Gln Val Leu Pro Cys Ser Phe Thr Thr Leu Pro Ala
325 330

WHAT IS CLAIMED IS:

1. Plasmid pHCV-162.
2. Plasmid pHCV-167.
3. Plasmid pHCV-168.
- 5 4. Plasmid pHCV-169.
5. Plasmid pHCV-170.
6. APP-HCV-E2 fusion protein expressed by a mammalian expression vector pHCV-162.
7. APP-HCV-E2 fusion protein expressed by a mammalian expression vector pHCV-167.
- 10 8. HGH-HCV-E2 fusion protein expressed by a mammalian expression vector pHCV-168.
9. HGH-HCV-E2 fusion protein expressed by a mammalian expression vector pHCV-169.
- 15 10. HGH-HCV-E2 fusion protein expressed by a mammalian expression vector pHCV-170.
11. A method for detecting HCV antigen or antibody in a test sample suspected of containing HCV antigen or antibody, wherein the improvement comprises contacting the test sample with a glycosylated HCV antigen produced in a mammalian expression system.
- 20 12. A method for detecting HCV antigen or antibody in a test sample suspected of containing HCV antigen or antibody, wherein the improvement comprises contacting the test sample with an antibody produced by using a glycosylated HCV antigen produced in a mammalian expression system.
- 25 13. The method of claim 12 wherein said antibody is a monoclonal antibody.
14. The method of claim 12 wherein said antibody is a polyclonal antibody.
15. A test kit for detecting the presence of HCV antigen or HCV antigen in a test sample suspected of containing said HCV antigen or antibody, comprising:
 - a container containing a glycosylated HCV antigen produced in a mammalian expression system.
- 30 16. The test kit of claim 15 further comprising an antibody produced by using a glycosylated HCV antigen produced in a mammalian expression system.
- 35

84

17. A test kit for detecting the presence of HCV antigen or HCV antigen in a test sample suspected of containing said HCV antigen or HCV antibody, comprising:

5 a container containing an antibody produced by using a glycosylated HCV antigen produced in a mammalian expression system.

18. The test kit of claim 17 wherein said antibody is a polyclonal antibody.

19. The test kit of claim 17 wherein said antibody is a monoclonal antibody.

10

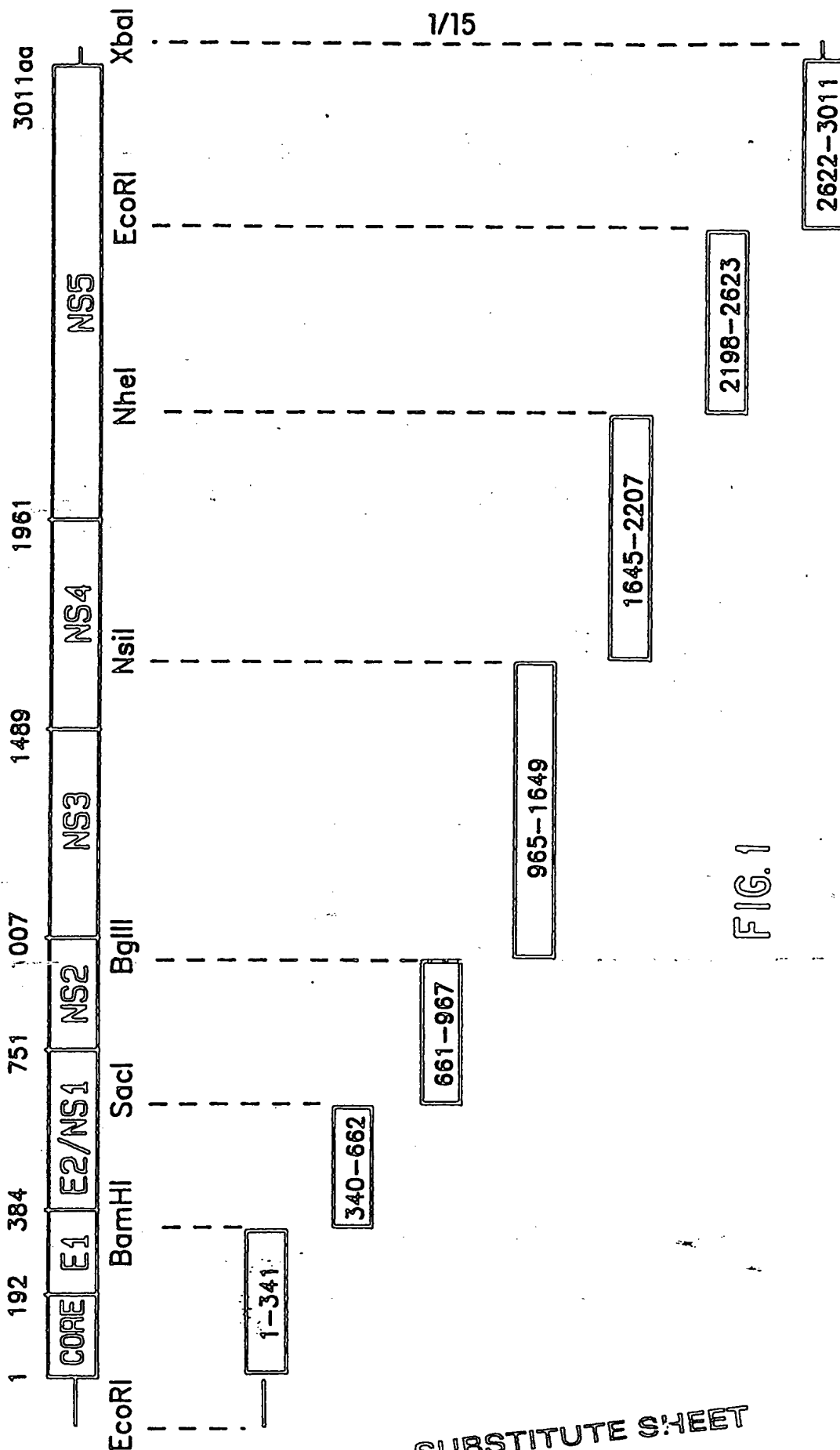


FIG. 1

SUBSTITUTE SHEET

2/15

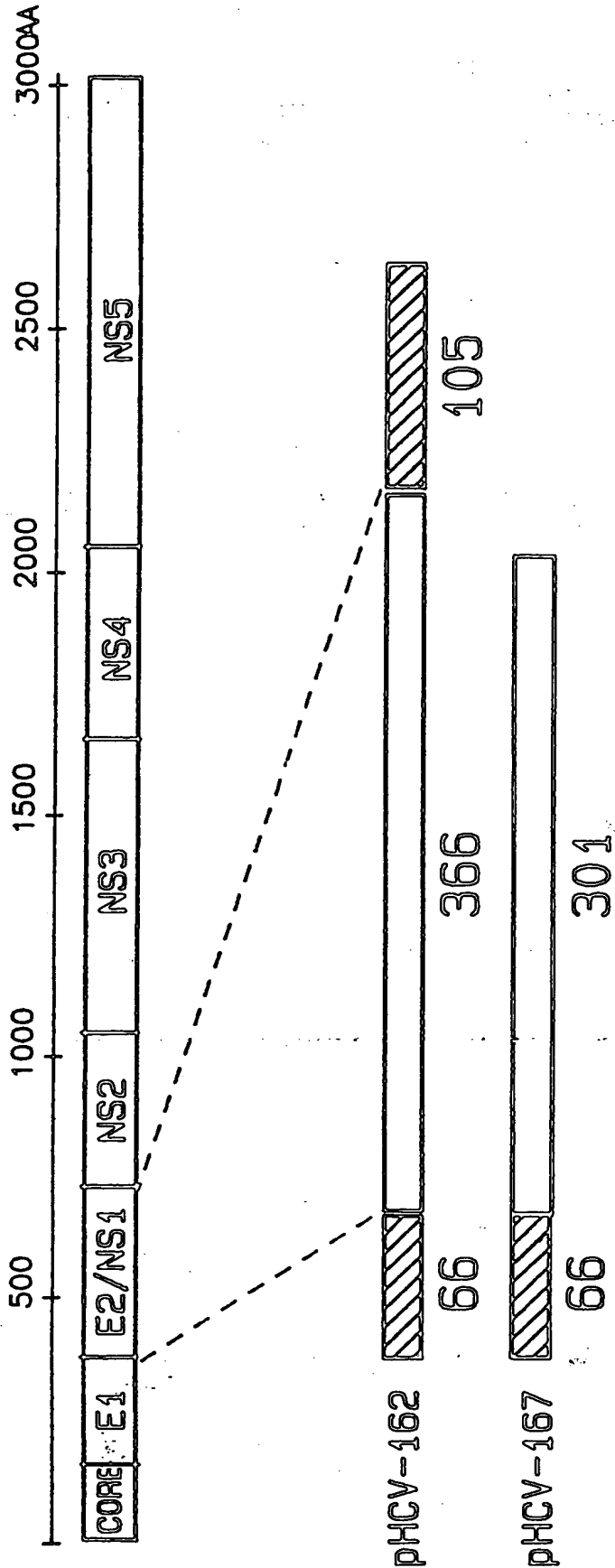


FIG. 2

HCV AA# 384-749 or
HCV AA# 384-684
FUSION TO APP PROTEIN
CMV PROMOTER
HEK CELLS

3/15

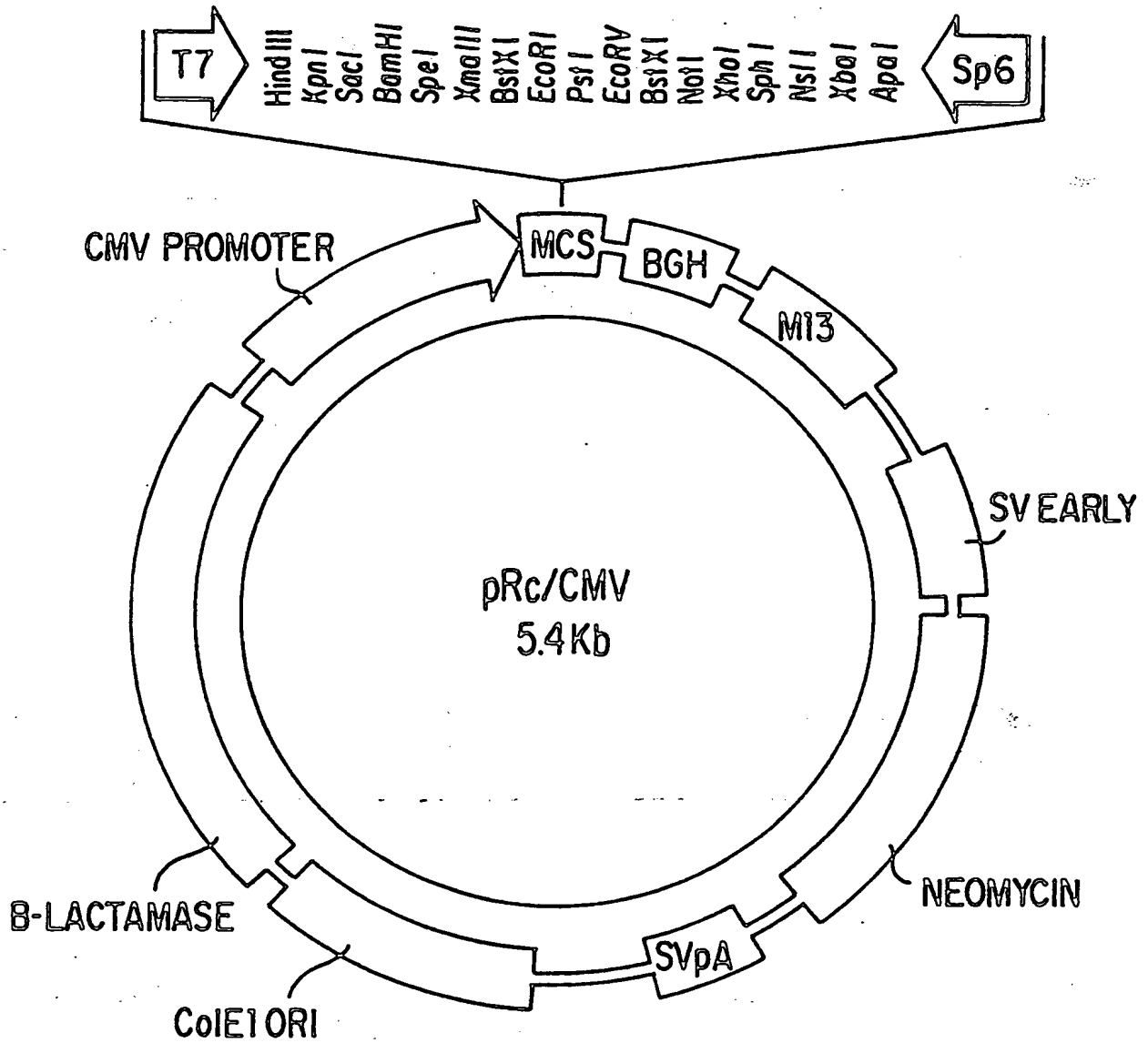


FIG. 3

4/15

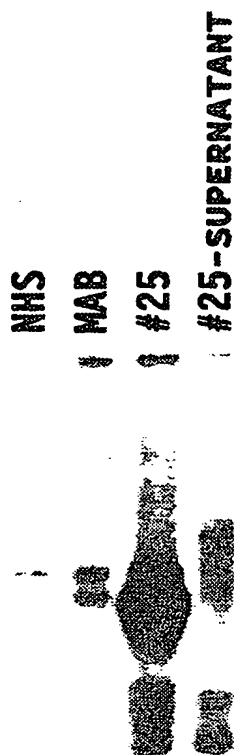


FIG. 4

5/15

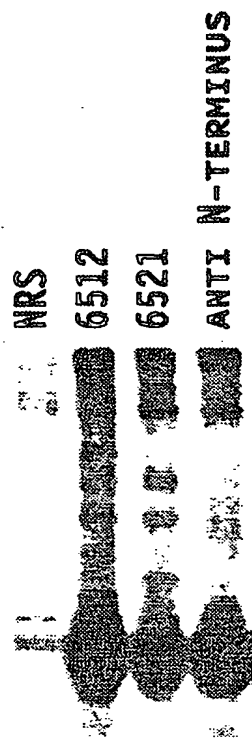


FIG. 5

6/15

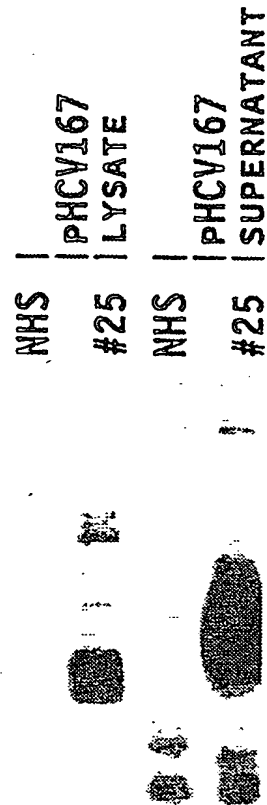


FIG. 6

7/15

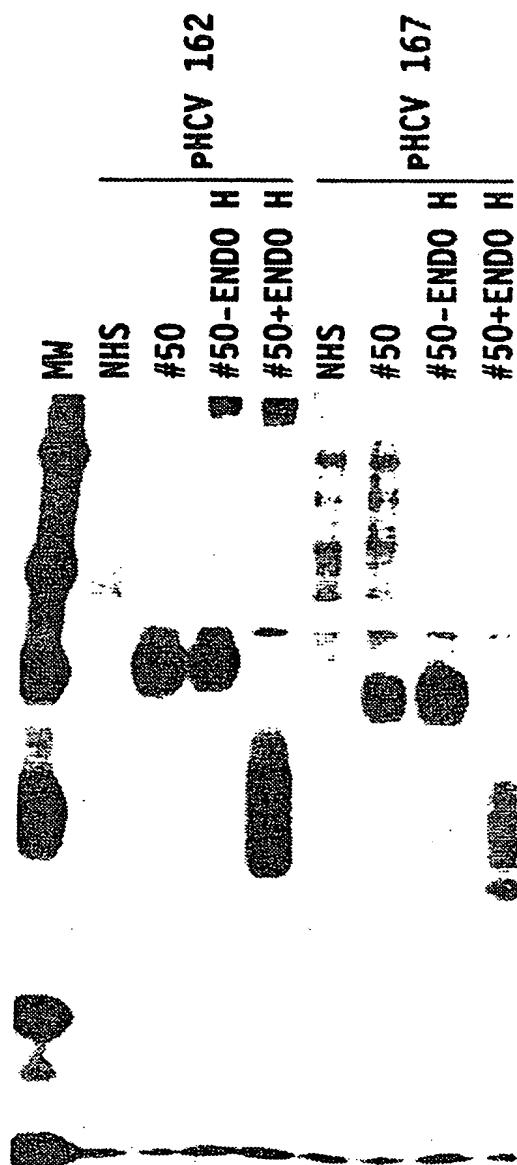


FIG. 7

8/15

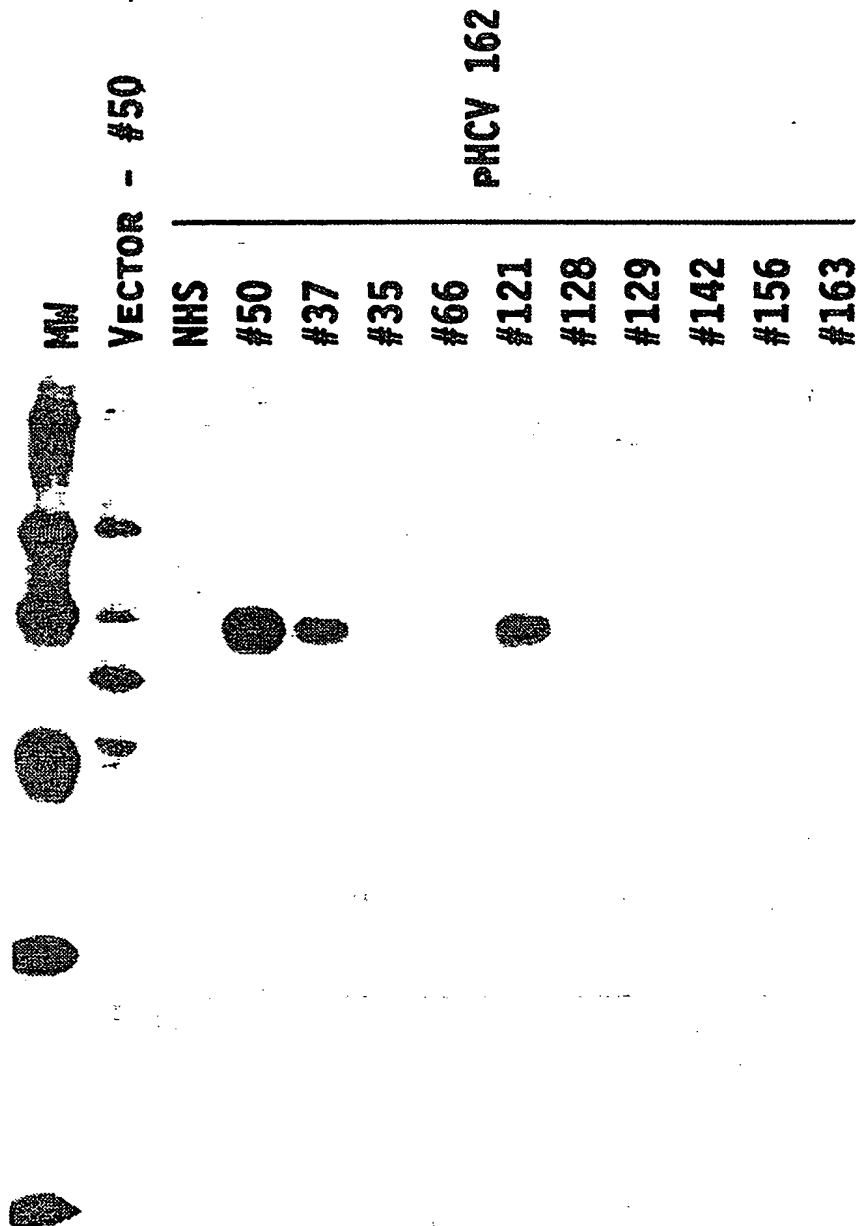


FIG. 8

9/15



MW
#50
410
435
441
476
496
560
589
620
622
623
633
639
641
648
649
657
666
672

FIG. 9**SUBSTITUTE SHEET**

10/15

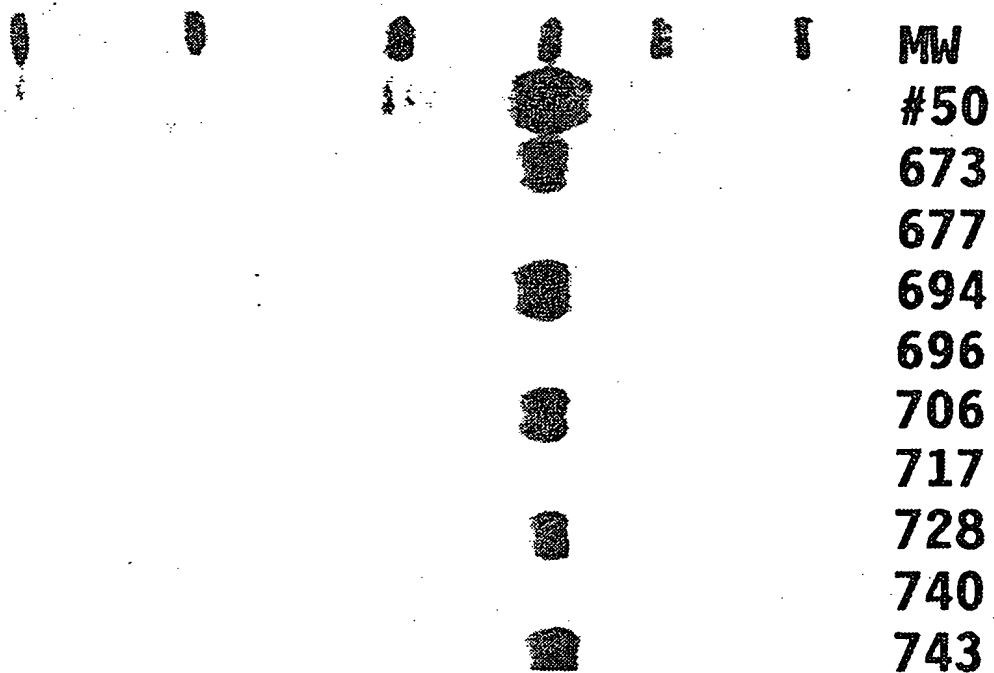


FIG. 10

11/15

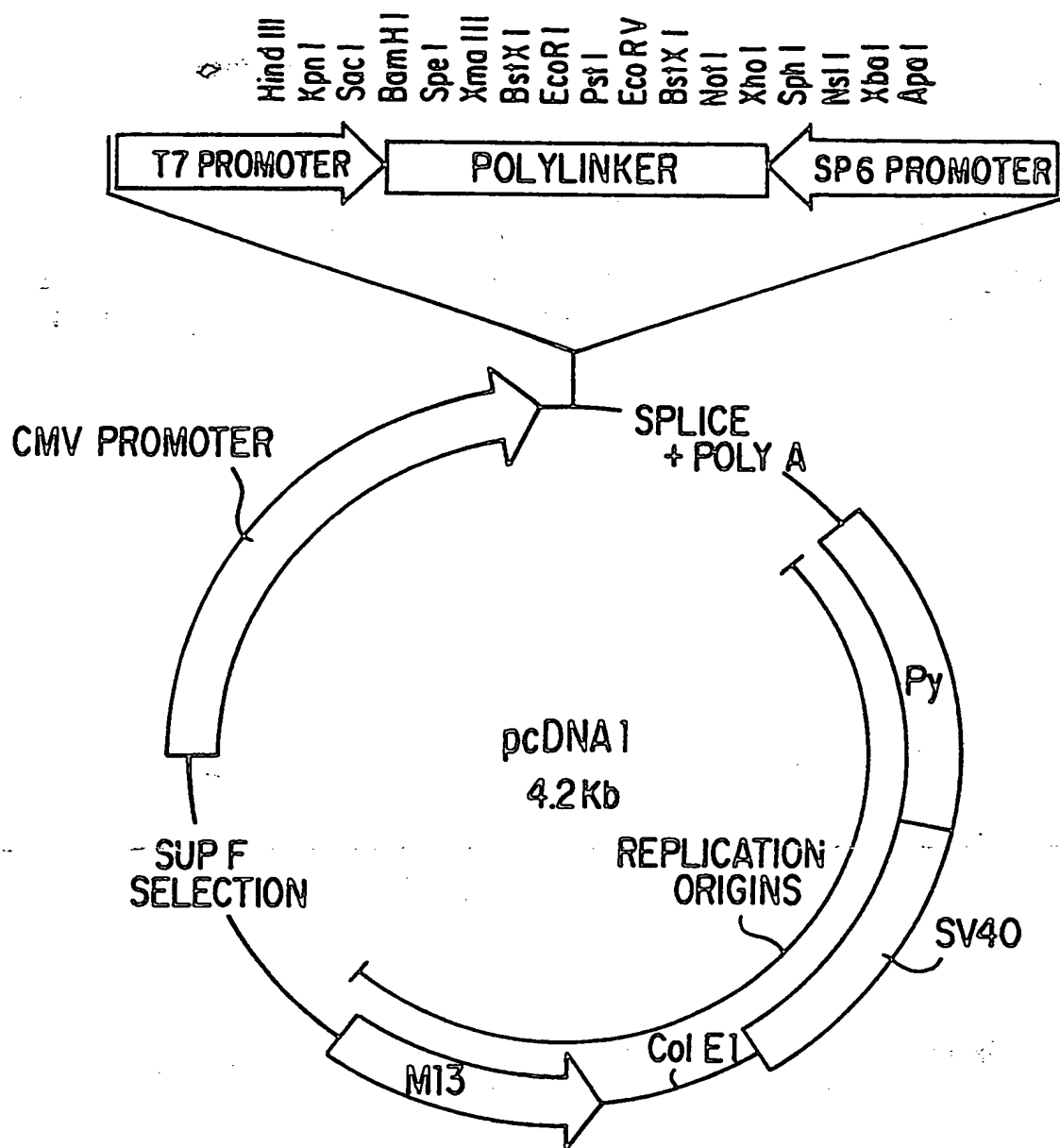
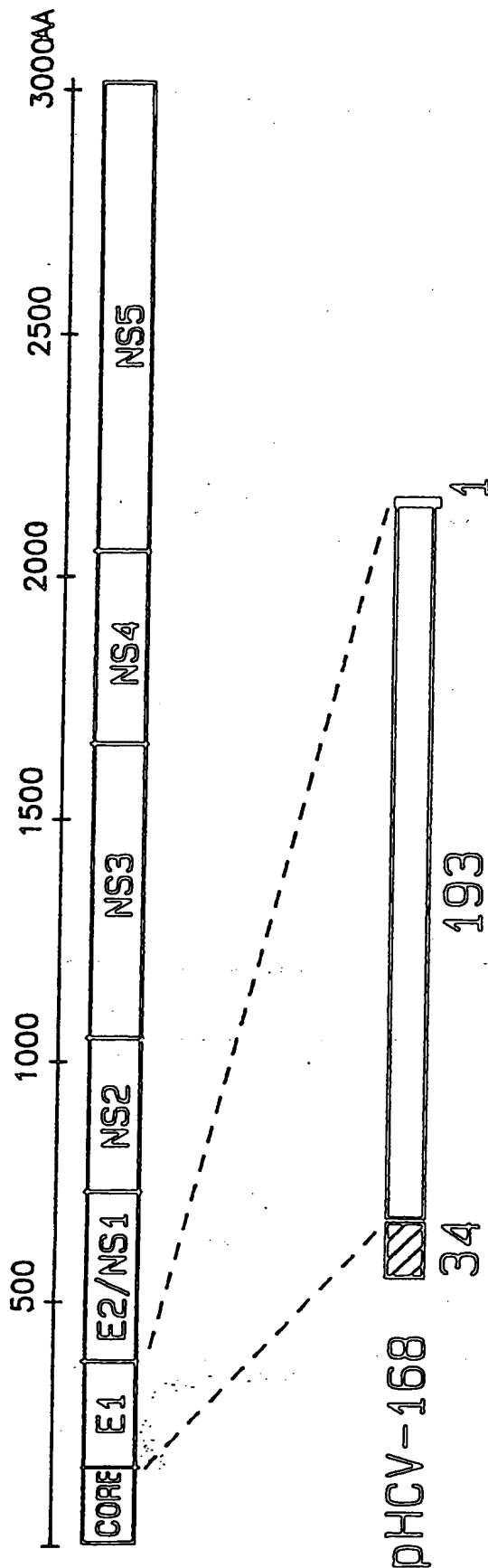


FIG. 11

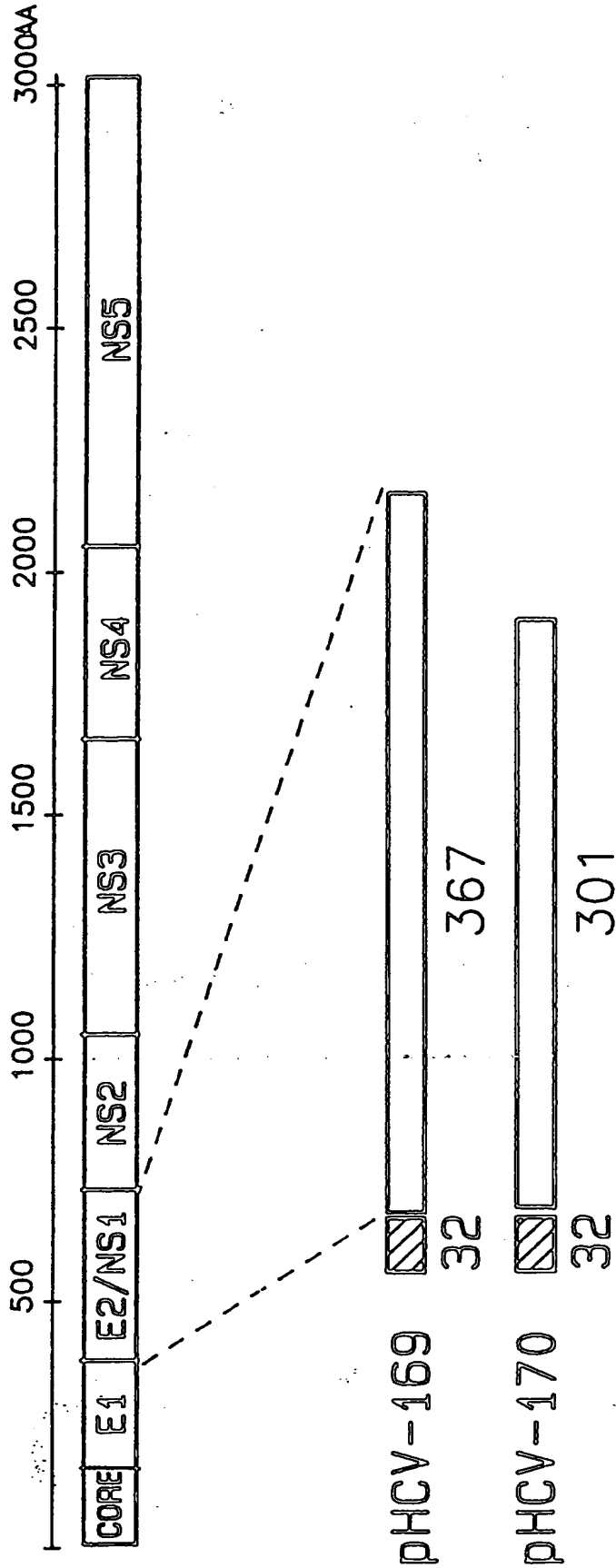
12/15



HCV AA# 192-384
 HGH SECRETION SIGNAL
 CMV PROMOTER
 HEK CELLS

FIG.12

13/15



HCV AA# 384-750 or
HCV AA# 384-684
HGH SECRETION SIGNAL
CMV PROMOTER
HEK CELLS

FIG.13

14/15

PHCV 168
LYSATE

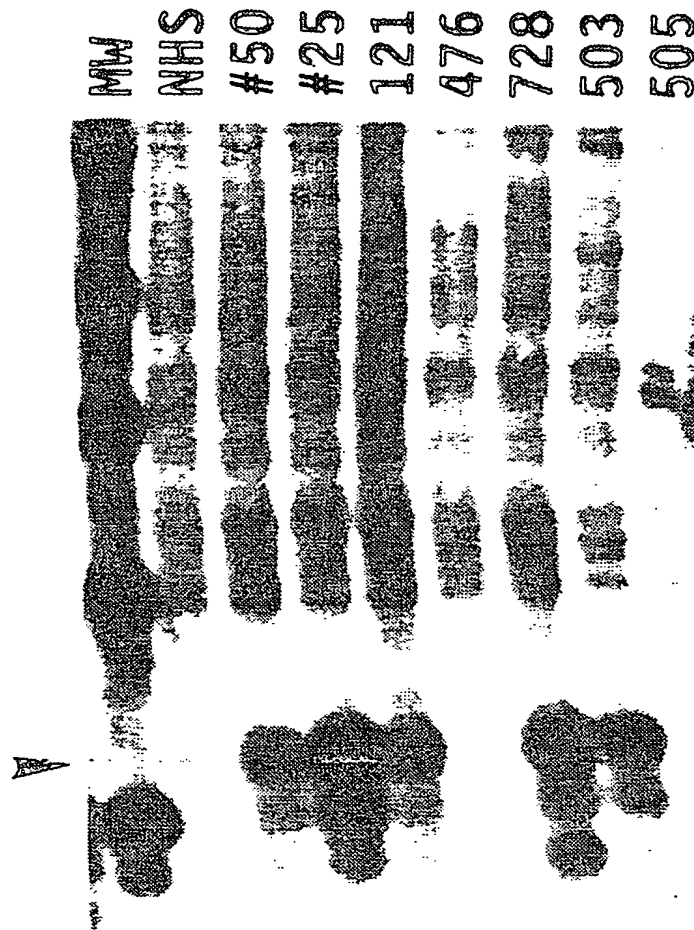


FIG. 14

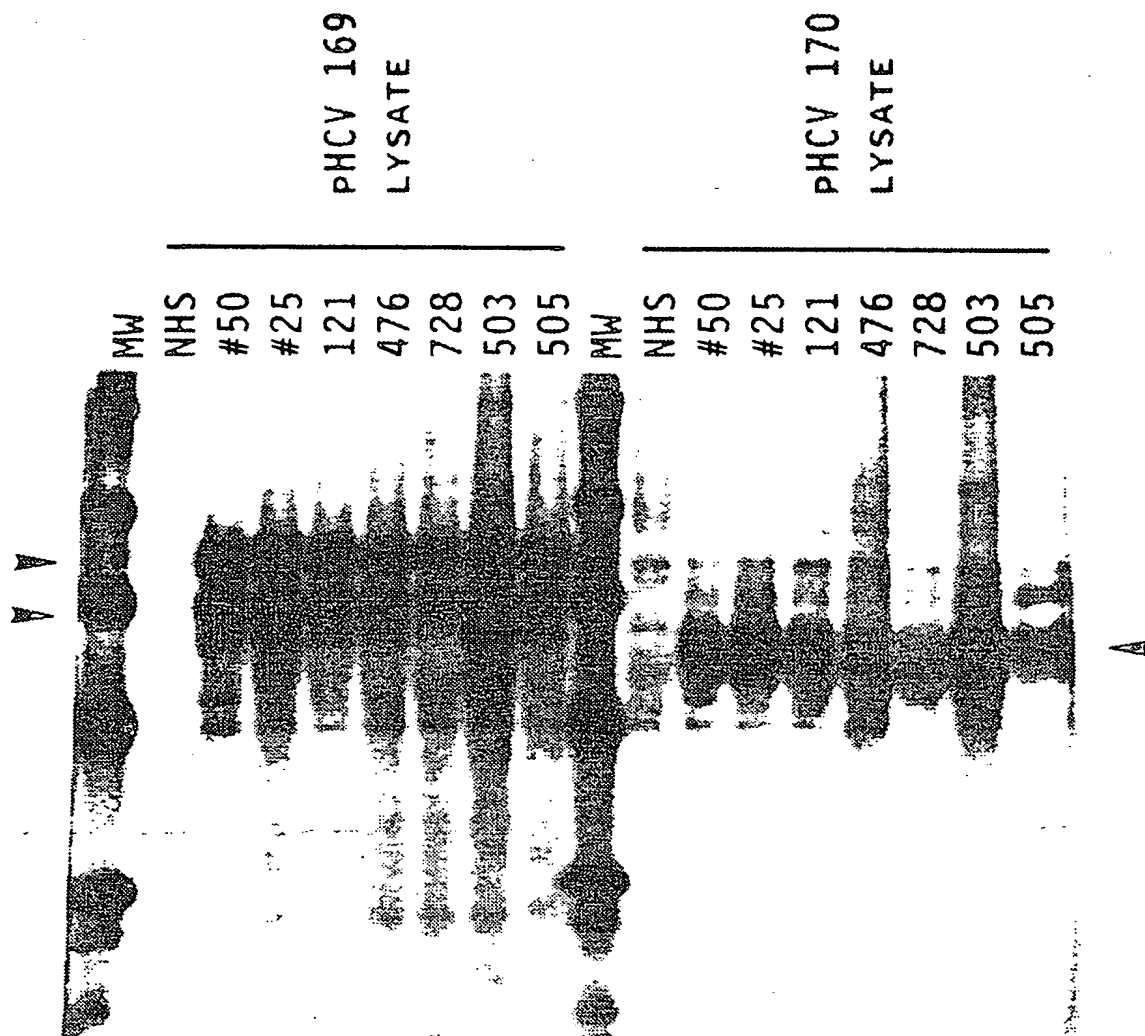


FIG. 15

A. CLASSIFICATION OF SUBJECT MATTER

IPC(S) : C12N 15/00; C12Q 1/70; C07K 15/00

US CL : 435/320.1, 5; 530/409

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/320.1, 69.3, 5, 7.1; 530/350, 409

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

PIR, SWISS-PROT, GENESEQ, GENBANK, WPI, CA, MEDLINE, APS

search terms: hepatitis C virus, HCV, fusion, amyloid precursor protein, human growth hormone, diagnosis?, kit

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Proceedings of the National Academy of Sciences USA, Volume 88, issued March 1991. Q.-L. Choo et al, "Genetic Organization and Diversity of the Hepatitis C Virus", pp. 2451-2455, see entire document.	1-18
Y	Journal of General Virology, Volume 72, issued October 1991, D. Kremsdorf et al., "Partial Nucleotide Sequence Analysis of a French Hepatitis C Virus: Implications for HCV Variability in the E2/NS1 Protein", pp. 2557-2561, see entire document.	1-18

☒ Further documents are listed in the continuation of Box C.
 ☐ See patent family annex.

A	document defining the general state of the art which is not considered to be part of particular relevance	*T*	later document published after the international filing date or priority date and not in conflict with the application but cited to underpin the principles or theory underlying the invention
E	earlier document published on or after the international filing date	*X*	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
L	document which may throw doubt on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*Y*	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
O	document referring to an oral disclosure, use, exhibition or other means	*Z*	document member of the same patent family
P	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 30 April 1993	Date of mailing of the international search report 11 MAY 1993
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. NOT APPLICABLE	Authorized officer D. BARND Telephone No. (703) 308-0196

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category ^a	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Journal of Virology, Volume 65, No. 3, issued March 1991, A. Takamizawa et al., "Structure and Organization of the Hepatitis C Virus Genome Isolated from Human Carriers", pp. 1105-1113, see entire document.	1-18
Y	Proceedings of the National Academy of Sciences USA, Volume 87, issued December 1990, N. Kato et al., "Molecular Cloning of the Human Hepatitis C Virus Genome from Japanese Patients with non-A, non-B Hepatitis", pp. 9524-9528, see entire document.	1-18
Y	Journal of General Virology, Volume 72, issued November 1991, H. Okamoto et al., "Nucleotide Sequence of the Genomic RNA of Hepatitis C Virus Isolated from a Human Carrier: Comparison with Reported Isolates for Conserved and Divergent Regions", pp. 2697-2704, see entire document.	1-18
Y	Gene, Volume 105, No. 2, issued 1991, J. Li et al., "Two French Genotypes of Hepatitis C Virus: Homology of the Predominant Genotype with the Prototype American Strain", pp. 167-172, see entire document.	1-18
Y,P	US, A, 5,106,726 (Wang) 21 April 1992, see entire document.	1-18
Y	EP, A, 0,318,216 (Houghton et al) 31 May 1989, see entire document.	1-18
Y	EP, A, 0,388,232 (Houghton et al) 19 September 1990, see entire document.	1-18
Y	GB, A, 2,212,511 (Houghton et al) 26 July 1989, see entire document.	1-18
Y	Cell, Volume 57, No. 1, issued 07 April 1989, A. Weidemann et al., "Identification, Biogenesis, and Localization of Precursors of Alzheimer's Disease A4 Amyloid Protein", pp. 115-126, see entire document.	1,2,6,7,11-18
Y	The Journal of Biological Chemistry, Volume 266, No. 29, issued 15 October 1991, D. E. Lowery et al., "Alzheimer's Amyloid Precursor Protein Produced by Recombinant Baculovirus Expression", pp. 19842-19850, see entire document.	1,2,6,7,11-18
Y	Vaccine, Volume 9, No. 8, issued August 1991, M. Kit et al., "Bovine Herpesvirus-1 (Infectious Bovine Rhinotracheitis Virus)-Based Viral Vector which Expresses Foot-and-Mouth Disease Epitopes", pp. 564-572, see entire document.	3-5,8-18